NUCLEIC ACID MOLECULES AND OTHER MOLECULES ASSOCIATED WITH SOYBEAN CYST NEMATODE RESISTANCE

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority under 35 U.S.C. §119(e) of U.S. Application No.

5   60/174,880, filed January 7, 2000, the disclosure of which is herein incorporated by reference in

its entirety.

FIELD OF THE INVENTION

The present invention is in the field of soybean genetics. More specifically, the invention

relates to nucleic acid molecules from regions of the soybean genome, which are associated with

10   soybean cyst nematode (SCN) resistance. The invention also relates to proteins encoded by such

nucleic acid molecules as well as antibodies capable of recognizing these proteins. The

invention also relates to nucleic acid markers from regions of the soybean genome, which are

associated with SCN resistance. Moreover, the invention relates to uses of such molecules,

including, transforming SCN sensitive soybean with constructs containing nucleic acid

15   molecules from regions in the soybean genome, which are associated with SCN resistance.

Furthermore, the invention relates to the use of such molecules in a plant breeding program.

BACKGROUND OF THE INVENTION

The soybean, *Glycine max* (L.) Merril (*Glycine max* or soybean), is one of the major

economic crops grown worldwide as a primary source of vegetable oil and protein (Sinclair and

20   Backman, *Compendium of Soybean Diseases*, 3rd Ed. APS Press, St. Paul, MN, p. 106. (1989)).

The growing demand for low cholesterol and high fiber diets has also increased soybean's

importance as a health food.

Prior to 1940, soybean cultivars were either direct releases of introductions brought from

Asia or pure line selections from genetically diverse plant introductions. The soybean plant was

25   primarily used as a hay crop in the early part of the 19th century. Only a few introductions were

large-seeded types useful for feed grain and oil production. From the mid 1930's through the 1960's, gains in soybean seed yields were achieved by changing the breeding method from evaluation and selection of introduced germplasm to crossing elite by elite lines. The continuous cycle of cross hybridizing the elite strains selected from the progenies of previous crosses

5   resulted in the modern day cultivars.

Over 10,000 soybean strains have now been introduced into the United States since the early 1900's (Bernard *et al., United States National Germplasm Collections.* In: L.D. Hil (ed.), World Soybean Research, pp. 286-289. Interstate Printers and Publ., Danville, Il. (1976)). A limited number of those introductions form the genetic base of cultivars developed from the

10   hybridization and selection programs (Johnson and Bernard, *The Soybean,* Norman Ed., Academic Press, N.Y., pp. 1-73 (1963)). For example, in a survey conducted by Specht and Williams, *Genetic Contributions,* Fehr eds. American Soil Association, Wisconsin, pp. 49-73 (1984), for the 136 cultivars released from 1939 to 1989, only 16 different introductions were the source of cytoplasm for 121 of that 136. Certain soybean strains are sensitive to one or more

15   pathogens. One economically important pathogen is SCN.

SCN accounts for roughly 40% of the total disease in soybean and can result in significant yield losses (up to 90%). SCN is the most destructive pest of soybean to date and accounts for an estimated yield loss of up to $809 million dollars annually. Currently, the most cost effective control measures are crop rotation and the use of host plant resistance. While

20   breeders have successfully developed SCN resistant soybean lines, breeding is both difficult and time consuming due to the complex and polygenic nature of resistance. The resistance is often race specific and does not provide stability over time due to changing SCN populations in the field. In addition, many of the resistant soybean varieties carry a significant yield penalty when grown in the absence of SCN.

25   SCN, *Heterodera glycines Ichinohe,* was identified on soybeans in the United States in 1954 at Castle Hayne, N.C. Winstead, *et al., Plant Dis. Rep. 39:9-11* (1955). Since its discovery the SCN has been recognized as one of the most destructive pests in soybean. It has been

reported in nearly all states in which soybeans are grown, and it causes major production

problems in several states, being particularly destructive in the Midwestern states. See generally:

Caldwell, *et al.*, *Agron. J. 52*:635-636 (1960); Rao-Arelli and Anand, *Crop. Sci. 28*:650-652,

(1988); Baltazar and Mansur, *Soybean Genet. Newsl. 19*:120-122 (1992); Concibido, *et al.*,

5   *Crop. Sci.*, (1993). For example, sensitive soybean cultivars had 5.7-35.8% lower seed yields

than did resistant cultivars on SCN race-3 infested sites in Iowa. (Niblack and Norton, *Plant Dis.*

76:943-948 (1992)).

Shortly after the discovery of SCN in the United States, sources of SCN resistance were

identified (Ross and Brim, *Plant Dis. Rep.* 41:923-924 (1957)). Some lines such as Peking and

10   Plant Introduction (PI) PI88788, were quickly incorporated into breeding programs. Peking

became widely used as a source of resistance due to its lack of agronomically undesirable traits,

with Pickett as the first SCN resistant cultivar released (Brim and Ross, *Crop Sci.* 6:305 (1966)).

The recognition that certain SCN resistant populations could overcome resistant cultivars lead to

an extensive screen for additional sources of SCN resistance. PI88788 emerged as a popular

15   source of race 3 and 4 resistance even though it had a cyst index greater than 10% (but less than

20%) against race 4, and Peking and its derivatives emerged as a popular source for races 1 and

3. PI437654 was subsequently identified as having resistance to all known races and its SCN

resistance was backcrossed into Forrest. Currently there are more than 130 PIs known to have

SCN resistance.

20   SCN race 3 is considered to be the prominent race in the Midwestern soybean producing

states. Considerable effort has been devoted to the genetics and breeding for resistance to race 3.

While both Peking and PI88788 are resistant to SCN race 3, classical genetics studies suggest

that they harbor different genes for race 3 resistance (Rao-Arelli and Anand, *Crop Sci. 28*:650-

652 (1988)). Crosses between PI88788(R) and Essex(S) segregate 9(R): 55(S) in the F_2

25   population and 1(R): 26(Seg): 37(S) families in the F_3 generation, suggesting that resistance to

race 3 in PI88788 is conditioned by one recessive and two dominant genes, where as Peking and

PI90763 resistance is conditioned by one dominant and two recessive genes. Based on

reciprocal crosses, Peking, Forrest, and PI90763 have genes in common for resistance to SCN

race 3 (Rao-Arelli and Anand, *Crop Sci., 28*:650-652 (1988)). A cross between Peking and

PI88788 segregates 13(R):3(S) in the F_2 generation, indicating a major difference between the

parents for race 3 resistance. Generation mean analysis based on four crosses between resistant

5   and sensitive genotypes; A20 (R), Jack (R), Cordell (R) and A2234 (S), suggests that an additive

genetic model is sufficient to explain most of the genetic variation of race 3 SCN resistance in

each cross, while the analysis of the pooled data indicates the presence of dominant effects as

well (Mansur, Carriquiry and Roa-Arelli, *Crop Sci. 33*:1249-1253 (1993)). This analysis further

indicates that race 3 resistance is probably under the genetic control of three, but not more than

10   four genes.

       RFLP analysis of segregating populations between resistant and sensitive lines; PI209332

(R), PI90763 (R), PI88788 (R), Peking (R) and Evan (S), identified a major SCN resistance QTL

(*rhg*1) which maps to linkage group G (Concibido *et al.*, *Theor Appl. Genet. 93*:234-241

(1996)). In this study, *rhg*1 explains 51.4% of the phenotypic variation in PI209322, 52.7% of

15   the variation in PI90763, 40.0% of the variation in PI88788 and 28.1% of the variation in Peking.

This major resistance QTL was assumed be one and the same in all of the mapping populations

employed. However, as pointed out by the authors, it is possible that the genomic interval

contains distinct but tightly linked QTLs. In a related study using PI209332 as the source of

resistance, Concibido *et al.*, *Crop Sci. 36*:1643-1650 (1996), show that a QTL on linkage group

20   G (*rhg*1) is effective against the three SCN races tested, explaining 35% of the phenotypic

variation to race 1, 50% of the variation to race 3, and 54% of the variation to race 6. In addition

to the major QTL on linkage group G, 4 other QTLs mapping to linkage groups D, J, L and K

were identified, with some of the resistance loci behaving in a race specific manner.

       Concibido *et al.* (*Crop Sci. 37*:258-264 (1997)) found significant association of marker

25   C006V to a major QTL on linkage group G (*rhg*1) and resistance to race 1, race 3 and race 6, in

Peking and PI90763 (Evan X Peking, Evan X PI90763) and races 3 and 6 in PI88788 (Evan X

PI88788), in agreement with the previous study based on the P209332 source of resistance

(Concibido *et al.*, *Crop Sci. 36*:1643-1650 (1996)). The resistance locus near C006V was effective against all races tested in all of the resistance sources. While statistically significant against all races, this locus accounts for different proportions of the total phenotypic variation with the races tested. For example, in PI90763 the resistance locus near C006V explains more than three times the phenotypic variation against race 1 than against race 3. The variability can be attributed to differences in the genetic backgrounds, variability among the SCN populations or may be a reflection of the limited size of the plant populations which were employed. This study further identified three additional independent SCN resistance QTLs; one near the RFLP marker A378H mapping to the opposite end of linkage group G from C006V (*rhg*1), one near the marker B032V-1 on linkage group J and a third linked to A280Hae-1 on linkage group N. Comparisons between the different SCN races indicated that some of the putative SCN QTLs behave in a race specific manner.

PI437654 was identified as having resistance to all known races. Based on analysis of 328 recombinant inbreed lines (RIL) derived from a cross between PI437654 and BSR101, Webb reported six QTLs associated with SCN resistance on linkage groups A2, C1, G, M, L25 and L26 (U.S. Patent 5,491,081). An allele on linkage group G, presumed to be *rhg*1, is involved with certain SCN races tested (races 1, 2, 3, 5 and 14), and has the largest reported phenotypic effect on resistance to every race. In contrast, the QTLs on linkage groups A2, C1, M, L25 and L26 act in a race specific manner. The QTL on linkage group L25 was reportedly involved with four of the five races, while the QTLs on linkage groups, A2, C1 and L26 were each involved in resistance to two of the five races (U.S. Patent 5,491,081). Webb further reports data that the resistance to any of the five races is likely to result from the combined effects of the QTL involved in each race (U.S. Patent 5,491,081).

Qui *et al.* (*Theor Appl Genet 98*:356-364 (1999)) screened 200 $F_{2:3}$ families derived from a cross between Peking and Essex and identified RFLP markers which are associated with SCN resistance QTLs on linkage groups B, E, I and H. The three QTLs on linkage groups B, E and H jointly account for 57.7% of the phenotypic variation to race 1, the QTLs on linkage groups H

and B account for 21.4% of the variation to race 3, while the QTLs on linkage groups I and E are associated with resistance to race 5 accounting for 14.0% of the phenotypic variation. In contrast to previous mapping studies which use Peking as the source of resistance, no significant association was detected to the *rhg*1 locus on linkage group G. The authors point out that the

5   marker Bng122, which has been shown to have significant linkage to *rhg*1, is not polymorphic in the population employed (Concibido *et al., Crop Sci. 36*:1643-1650 (1996)).

It has been reported that the *rhg*1 locus on linkage group G is necessary for the development of resistance to any of the SCN races. There have been efforts to develop molecular markers to identify breeding lines harboring the *rhg*1 SCN resistant allele. One of the

10   most commonly used markers for marker assisted selection (MAS) of *rhg*1 is an SSR locus that co-segregates and maps roughly 0.4 cM from *rhg*1. This SRR marker, BARC-Satt_309 is able to distinguish most, if not all, of the SCN sensitive genotypes from those harboring *rhg*1 from important sources of resistance such as Peking and PI437654. Two simple sequence repeat markers have been reported that can be used to select for SCN resistance at the *rhg*1 locus

15   (Concibido *et al., Theor Appl Genet* 99: 811-818 (1999)). Satt_309 was also effective in distinguishing SCN resistant sources PI88788 and PI209332 in many, but not all, sensitive genotypes. In particular, Satt_309 can not be used for MAS in populations developed from "typical" southern US cultivars (*e.g.*, Lee, Bragg and Essex) crossed with resistance sources PI88788 or PI209332.

20   Matson and Williams have reported a dominant SCN resistance locus, *Rhg*4, which is tightly linked to the '*i*' locus on linkage group A2 (Matson and Williams, *Crop Sci. 5*:447 (1965)). The QTL reported by Webb on linkage group A2 maps near the '*i*' locus and is considered to be *Rhg*4 (U.S. Patent 5,491,081). Webb concludes that only two loci on linkage groups A2 (*Rhg*4) and G (*rhg*1) explain the genetic variation to race 3.

SUMMARY OF THE INVENTION

The present invention includes and provides a method for the production of a soybean plant having an *rhg*1 SCN resistant allele comprising: (A) crossing a first soybean plant having an *rhg*1 SCN resistant allele with a second soybean plant having an *rhg*1 SCN sensitive allele to

5   produce a segregating population; (B) screening the segregating population for a member having an *rhg*1 SCN resistant allele with a first nucleic acid molecule capable of specifically hybridizing to linkage group G, wherein the first nucleic acid molecule specifically hybridizes to a second nucleic acid molecule that is linked to the *rhg*1 SCN resistant allele; and, (C) selecting the member for further crossing and selection.

10   The present invention includes and provides a method of investigating an *rhg*1 haplotype of a soybean plant comprising: (A) isolating nucleic acid molecules from the soybean plant; (B) determining the nucleic acid sequence of an *rhg*1 allele or part thereof; and, (C) comparing the nucleic acid sequence of the *rhg*1 allele or part thereof to a reference nucleic acid sequence. The present invention includes and provides a method of introgressing SCN resistance or partial

15   SCN resistance into a soybean plant comprising: performing marker assisted selection of the soybean plant with a nucleic acid marker, wherein the nucleic acid marker specifically hybridizes with a nucleic acid molecule having a first nucleic acid sequence that is physically linked to a second nucleic acid sequence that is located on linkage group G of soybean A3244, wherein the second nucleic acid sequence is within 500 kb of a third nucleic acid sequence which is capable

20   of specifically hybridizing with the nucleic acid sequence of SEQ ID NO: 5, 6, complements thereof, or fragments thereof having at least 15 nucleotides; and, selecting the soybean plant based on the marker assisted selection.

The present invention includes and provides a method for the production of a soybean plant having an *Rhg*4 SCN resistant allele comprising: (A) crossing a first soybean plant having an

25   *Rhg*4 SCN resistant allele with a second soybean plant having an *Rhg*4 SCN sensitive allele to produce a segregating population; (B) screening the segregating population for a member having an *Rhg*4 SCN resistant allele with a first nucleic acid molecule capable of specifically

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hybridizing to linkage group A2, wherein the first nucleic acid molecule specifically hybridizes to a second nucleic acid molecule linked to the *Rhg*4 SCN resistant allele; and, (C) selecting the member for further crossing and selection.

The present invention includes and provides a method of investigating an *Rhg*4 haplotype

5   of a soybean plant comprising: (A) isolating nucleic acid molecules from the soybean plant; (B) determining the nucleic acid sequence of an *Rhg*4 allele or part thereof; and (C) comparing the nucleic acid sequence of the *Rhg*4 allele or part thereof to a reference nucleic acid sequence.

The present invention includes and provides a method of introgressing SCN resistance or partial SCN resistance into a soybean plant comprising: performing marker assisted selection of

10 the soybean plant with a nucleic acid marker, wherein the nucleic acid marker specifically hybridizes with a nucleic acid molecule having a first nucleic acid sequence that is physically linked to a second nucleic acid sequence that is located on linkage group A2 of soybean A3244, wherein the second nucleic acid sequence is within 500 kb of a third nucleic acid sequence which specifically hybridizes with the nucleic acid sequence of SEQ ID NO: 7, complements thereof, or

15 fragments thereof having at least 15 nucleotides; and, selecting the soybean plant based on the marker assisted selection.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, 8-23, 28-43, complements thereof, and fragments of either.

20 The present invention includes and provides a substantially purified first nucleic acid molecule with nucleic acid sequence which specifically hybridizes to a second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NOs: 5, 6, 8-23, 28-43.

The present invention includes and provides a substantially purified nucleic acid

25 molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7, 44-47, and 50-53, complements thereof, and fragments of either.

The present invention includes and provides a substantially purified first nucleic acid molecule with nucleic acid sequence which specifically hybridizes to a second nucleic acid

molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NOs: 50-53.

The present invention includes and provides a substantially purified protein or fragment thereof comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5   1097, 1098, and 1100-1115 and fragments thereof.

The present invention includes and provides a substantially purified protein or fragment thereof comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 1099, and 1116-1119 and fragments thereof.

The present invention includes and provides a transformed plant having a nucleic acid 10   molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; (B) a structural nucleic acid molecule encoding a protein or fragment thereof comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1097, 1100, 1098, 1101, 1102-1115; and (C) a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of 15   polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention includes and provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; (B) a structural nucleic acid molecule encoding a protein or fragment thereof comprising an amino acid sequence selected from the group 20   consisting of SEQ ID NOs: 1099, 1116-1119; and (C) a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention includes and provides a transgenic seed having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions to cause the 25   production of a mRNA molecule; (B) a structural nucleic acid molecule encoding a protein or fragment thereof comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1097, 1100, 1098, 1101, 1102-1115; and (C) a 3' non-translated sequence that functions to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention includes and provides a transgenic seed having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions to cause the production of a mRNA molecule; (B) a structural nucleic acid molecule encoding a protein or fragment thereof comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1099, 1116-1119; and (C) a 3' non-translated sequence that functions to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

DESCRIPTION OF THE FIGURES

Figure 1 is an amino acid sequence alignment of the leucine rich repeat domain of *rhg*1.

Figure 2 is an amino acid sequence alignment of the leucine rich repeat domain of *Rhg*4.

DESCRIPTION OF THE SEQUENCE LISTINGS

The following sequence listings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these sequences in combination with the detailed description presented herein.

SEQ ID NOs: 1-7 and 1097-1099 all refer to sequences from the line A3244.

SEQ ID NO: 1 is sequence ID 515O02_region_G2 from line A3244, and is adjacent to the contig containing *rhg*1.

SEQ ID NO: 2 is sequence ID 240O17_region_G3 from line A3244, and contains the *rhg*1, *v.1* four exon gene at coding coordinates 45163-45314, 45450-45509, 46941-48763, 48975-49573. The amino acid translation for SEQ ID NO: 2 is SEQ ID NO: 1097.

SEQ ID NO: 3 is sequence ID 240O17_region_G3 from line A3244, and contains the *rhg*1, *v.2* two exon gene at coding coordinates 46798-48763 and 48975-49573. The amino acid translation for SEQ ID NO: 3 is SEQ ID NO: 1098.

SEQ ID NO: 4 is sequence ID 318O13_region_A3 from line A3244, contains the *Rhg4* gene at coding coordinates 111805-113968 and 114684-115204, and has an amino acid translation of SEQ ID NO: 1099.

SEQ ID NO: 5 is sequence ID 240O17_region_G3_8_mRNA, and comprises the two *rhg*1, *v.2* exons from the coding sequence portion of SEQ ID NO: 3.

SEQ ID NO: 6 is sequence ID 240O17_region_G3_8_cds, and comprises the four *rhg*1, *v.1* exons from the coding sequence portion of SEQ ID NO: 2.

SEQ ID NO: 7 is sequence ID 318O13_region_A3_17_cds, and comprises the *Rhg4* coding sequence portion from SEQ ID NO: 4.

SEQ ID NOs: 8-43 and 1100-1115 all refer to *rhg*1 sequences.

SEQ ID NO: 8 is sequence ID rhg1_A3244_amplicon from line A3244, contains four *rhg*1, *v.1* exons at coding coordinates 113-264, 400-459, 1891-3713, and 3925-4523, and has an amino acid translation of SEQ ID NO: 1100 and 1097.

SEQ ID NO: 9 is sequence ID rhg1_A3244_amplicon, contains two *rhg*1, *v.2* exons at coding coordinates 1748-3713 and 3925-4523 and has an amino acid translation of SEQ ID NO: 1101 and 1098.

SEQ ID NO: 10 is sequence ID rhg1_peking_amplicon from the line peking, contains four *rhg*1, *v.1* exons at coding coordinates 113-264, 400-459, 1888-3710, and 3903-4501, and has an amino acid translation of SEQ ID NO: 1102.

SEQ ID NO: 11 is sequence ID rhg1_peking_amplicon, contains two *rhg*1, *v.2* exons at coding coordinates 1745-3710 and 3903-4501, and has an amino acid translation of SEQ ID NO: 1103.

SEQ ID NO: 12 is sequence ID rhg1_toyosuzu_amplicon from the line toyosuzu, contains four *rhg*1, *v.1* exons at coding coordinates 113-264, 400-459, 1890-3712, and 3924-4522, and has an amino acid translation of SEQ ID NO: 1104.

SEQ ID NO: 13 is sequence ID rhg1_toyosuzu_amplicon, contains two *rhg1*, *v.2* exons at coding coordinates 1747-3712 and 3924-4522, and has an amino acid translation of SEQ ID NO: 1105.

SEQ ID NO: 14 is sequence ID rhg1_will_amplicon from the line will, contains four *rhg1*, *v.1* exons at coding coordinates 113-264, 400-459, 1891-3713, and 3925-4523, and has an amino acid translation of SEQ ID NO: 1106.

SEQ ID NO: 15 is sequence ID rhg1_will_amplicon, contains two *rhg1*, *v.2* exons at coding coordinates 1748-3713 and 3925-4523, and has an amino acid translation of SEQ ID NO: 1107.

SEQ ID NO: 16 is sequence ID rhg1_a2704_amplicon from the line A2704, contains four *rhg1*, *v.1* exons at coding coordinates 113-264, 400-459, 1891-3713, and 3925-4523, and has an amino acid translation of SEQ ID NO: 1108.

SEQ ID NO: 17 is sequence ID rhg1_a2704_amplicon, contains two *rhg1*, *v.2* exons at coding coordinates 1748-3713 and 3925-4523, and has an amino acid translation of SEQ ID NO: 1109.

SEQ ID NO: 18 is sequence ID rhg1_noir_amplicon from the line noir, contains four *rhg1*, *v.1* exons at coding coordinates 113-264, 400-459, 1876-3698, and 3910-4508, and has an amino acid translation of SEQ ID NO: 1110.

SEQ ID NO: 19 is sequence ID rhg1_noir_amplicon, contains two *rhg1*, *v.2* exons at coding coordinates 1733-3698 and 3910-4508, and has an amino acid translation of SEQ ID NO: 1111.

SEQ ID NO: 20 is sequence ID rhg1_lee_amplicon from the line lee, contains four *rhg1*, *v.1* exons at coding coordinates 113-264, 400-459, 1876-3698, and 3910-4508, and has an amino acid translation of SEQ ID NO: 1112.

SEQ ID NO: 21 is sequence ID rhg1_lee_amplicon, contains two *rhg1*, *v.2* exons at coding coordinates 1733-3698 and 3910-4508, and has an amino acid translation of SEQ ID NO: 1113.

SEQ ID NO: 22 is sequence ID rhg1_pi200499_amplicon from the line PI200499, contains four *rhg1, v.1* exons at coding coordinates 113-264, 400-459, 1876-3698, and 3910-4508, and has an amino acid translation of SEQ ID NO: 1114.

SEQ ID NO: 23 is sequence ID rhg1_ pi200499_amplicon, contains two *rhg1, v.2* exons at coding coordinates 1733-3698 and 3910-4508, and has an amino acid translation of SEQ ID NO: 1115.

SEQ ID NO: 24 is sequence ID 240O17_region_G3_forward_1, is a primer that hybridizes to coordinates 45051-45077 on contig 240017_region_G3 before the start codon, and can be used with SEQ ID NO: 25.

SEQ ID NO: 25 is sequence ID 240O17_region_G3_reverse_1, is a primer that hybridizes to coordinates 47942-47918 on contig 240017_region_G3, and can be used with SEQ ID NO: 24.

SEQ ID NO: 26 is sequence ID 240O17_region_G3_forward_2, is a primer that hybridizes to coordinates 47808-47831 on contig 240017_region_G3, and can be used with SEQ ID NO: 27.

SEQ ID NO: 27 is sequence ID 240O17_region_G3_reverse_2, is a primer that hybridizes to coordinates 49553-49531 of contig 240017_region_G3 prior to the stop codon, and can be used with SEQ ID NO: 26.

Primers given by SEQ ID NOs: 24-27 are used to create the amplicons of SEQ ID NOs: 8-23. The final 22 bases are added to the actual amplicons in order to simulate the rest of the gene to the stop codon, in order to allow complete translation.

SEQ ID NO: 28 is sequence ID rhg1_A3244_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 8.

SEQ ID NO: 29 is sequence ID rhg1_peking_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 10.

SEQ ID NO: 30 is sequence ID rhg1_toyosuzu_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 12.

SEQ ID NO: 31 is sequence ID rhg1_will_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 14.

SEQ ID NO: 32 is sequence ID rhg1_a2704_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 16.

5 SEQ ID NO: 33 is sequence ID rhg1_noir_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 18.

SEQ ID NO: 34 is sequence ID rhg1_lee_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 20.

SEQ ID NO: 35 is sequence ID rhg1_pi200499_amplicon_cds, which is the coding

10 sequence portion of SEQ ID NO: 22.

SEQ ID NO: 36 is sequence ID rhg1_A3244_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 9.

SEQ ID NO: 37 is sequence ID rhg1_peking_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 11.

15 SEQ ID NO: 38 is sequence ID rhg1_toyosuzu_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 13.

SEQ ID NO: 39 is sequence ID rhg1_will_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 15.

SEQ ID NO: 40 is sequence ID rhg1_a2704_amplicon_cds_2, which is the coding

20 sequence portion of SEQ ID NO: 17.

SEQ ID NO: 41 is sequence ID rhg1_noir_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 19.

SEQ ID NO: 42 is sequence ID rhg1_lee_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 21.

25 SEQ ID NO: 43 is sequence ID rhg1_pi200499_amplicon_cds_2, which is the coding sequence portion of SEQ ID NO: 23.

SEQ ID NOs: 44-53 and 1116-1119 all refer to *Rhg*4 sequences

SEQ ID NO: 44 is sequence ID rhg4_a3244_amplicon from the line A3244, contains *Rhg4* at coding coordinates 79-2242 and 2958-3478, is made using SEQ ID NOs: 48 and 49, and has an amino acid translation of SEQ ID NO: 1116 and 1099.

SEQ ID NO: 45 is sequence ID rhg4_Minsoy_amplicon from the line Minsoy, contains *Rhg4* at coding coordinates 79-2242 and 2958-3478, is made using SEQ ID NOs: 48 and 49, and has an amino acid translation of SEQ ID NO: 1117.

SEQ ID NO: 46 is sequence ID rhg4_Jack_amplicon from the line Jack, contains *Rhg4* at coding coordinates 79-2242 and 2958-3478, is made using SEQ ID NO: 48 and 49, and has an amino acid translation of SEQ ID NO: 1118.

SEQ ID NO: 47 is sequence ID rhg4_peking_amplicon from the line Peking, contains *Rhg4* at coding coordinates 79-2242 and 2958-3478, is made using SEQ ID NOs: 48 and 49, and has an amino acid translation of SEQ ID NO: 1119.

SEQ ID NO: 48 is sequence ID 318O13_region_A3_forward, hybridizes to coordinates 111727-111756 of contig 318O13_region_A3, and is a primer used with SEQ ID NO: 49 to create *Rhg4* amplicons.

SEQ ID NO: 49 is sequence ID 318O13_region_A3_reverse, hybridizes to coordinates 115206-115177 of contig 318O13_region_A3, and is a primer used with SEQ ID NO: 48 to create *Rhg4* amplicons.

SEQ ID NO: 50 is sequence ID rhg4_A3244_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 44.

SEQ ID NO: 51 is sequence ID rhg4_Minsoy_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 45.

SEQ ID NO: 52 is sequence ID rhg4_Jack_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 46.

SEQ ID NO: 53 is sequence ID rhg4_peking_amplicon_cds, which is the coding sequence portion of SEQ ID NO: 47.

SEQ ID NO: 1120 is sequence ID consensusLRR, which is a consensus sequence for the LRR repeats shown in Figures 1 and 2.

SEQ ID NO: 1121 is sequence ID rhg1LRR, which is the amino acid sequence of the LRR domain shown in Figure 1.

5   SEQ ID NO: 1122 is sequence ID Rhg4LRR, which is the amino acid sequence of the LRR domain shown in Figure 2.

SEQ ID NO: 1123 is sequence ID 240017_region_G3_forward_1_b, which is an alternate primer that hybridizes to coordinates 45046-45072 on contig 240017_region_G3 before the start codon, and which can be used with SEQ ID NO: 25.

10   Table 1 below provides further information on the sequences described herein.

In table 1, for all rows, "Seq Num" refers to the corresponding SEQ ID NO in the sequence listing.

For rows with SEQ ID NOs: 1-53 and 1120-1123 "Seq ID" refers to the name of the SEQ ID NO given in the "Seq Num" column.

15   For rows with SEQ ID NOs: 2-4, 8-23, and 44-47 "Coding Sequence" refers to the coordinates of the coding portion of the SEQ ID NO given in the "Seq Num" column, and "AA" refers to the SEQ ID NO that is the amino acid translation of the SEQ ID NO given in the "Seq Num" column.

For rows with SEQ ID NOs: 24-27 and 1123, "Primer location on 240017_region_G3"

20   refers to the coordinates of the 240017_region_G3 contig to which the SEQ ID NO given in the "Seq Num" column hybridizes.

For rows with SEQ ID NOs: 48 and 49, "Primer location on 318013_region_A3" refers to the coordinates of the 318013_region_A3 contig to which the SEQ ID NO given in the "Seq Num" column hybridizes.

25   For rows with SEQ ID NOs: 54-400, "Seq ID" refers to the names of amplicon sequences. Within the Seq ID is the "__" (double length underscore) symbol. The name before

this symbol refers to the name of the contig in which the amplicon is found, and the numbers

after this symbol refer to the nucleotide location of the SSR on the contig.

For rows with SEQ ID NOs: 401-1096, "Seq ID" refers to the names of primer sequences

used in PCR to generate the amplicon sequences in table 1. For these rows, the "Seq ID" name

5   contains the same name as the amplicon that is generated by the pair of primers of which the

SEQ ID NO referred to in the first column is a member. The "Seq ID" name also contains either

"Forward" or "Reverse," which indicates the orientation of the primer. For these sequences,

"location of primer on contig start" and "location of primer on contig end" refer, respectively, to

the first and last base number of the contig on which the primer aligns.

10

TABLE 1

Seq Num	Seq ID		
1	515O02_region_G2		

Seq Num	Seq ID	Coding Sequence	AA No.
2	240O17_region_G3	45163-45314,45450-45509,46941-48763,48975-49573	1097
3	240O17_region_G3	46798-48763,48975-49573	1098
4	318O13_region_A3	111805-113968,114684-115204	1099

Seq Num	Seq ID		
5	240O17_region_G3_8_mRNA		
6	240O17_region_G3_8_cds		
7	318O13_region_A3_17_cds		

Seq Num	Seq ID	Coding Sequence	AA No.
8	rhg1_A3244_amplicon	113-264,400-459,1891-3713,3925-4523	1100
9	rhg1_A3244_amplicon	1748-3713,3925-4523	1101
10	rhg1_peking_amplicon	113-264,400-459,1888-3710,3903-4501	1102
11	rhg1_peking_amplicon	1745-3710,3903-4501	1103
12	rhg1_toyosuzu_amplicon	113-264,400-459,1890-3712,3924-4522	1104
13	rhg1_toyosuzu_amplicon	1747-3712,3924-4522	1105
14	rhg1_will_amplicon	113-264,400-459,1891-3713,3925-4523	1106
15	rhg1_will_amplicon	1748-3713,3925-4523	1107
16	rhg1_a2704_amplicon	113-264,400-459,1891-3713,3925-4523	1108
17	rhg1_a2704_amplicon	1748-3713,3925-4523	1109
18	rhg1_noir_amplicon	113-264,400-459,1876-3698,3910-4508	1110
19	rhg1_noir_amplicon	1733-3698,3910-4508	1111
20	rhg1_lee_amplicon	113-264,400-459,1876-3698,3910-4508	1112
21	rhg1_lee_amplicon	1733-3698,3910-4508	1113
22	rhg1_pi200499_amplicon	113-264,400-459,1876-3698,3910-4508	1114
23	rhg1_pi200499_amplicon	1733-3698,3910-4508	1115

Seq Num	Seq ID	Primer location on 240O17_region_G3	
24	240O17_region_G3_forward_1	45051-45077	
25	240O17_region_G3_reverse_1	47942-47918	
26	240O17_region_G3_forward_2	47808-47831	
27	240O17_region_G3_reverse_2	49553-49531	

Seq Num	Seq ID		
28	rhg1_A3244_amplicon_cds		
29	rhg1_peking_amplicon_cds		
30	rhg1_toyosuzu_amplicon_cds		
31	rhg1_will_amplicon_cds		
32	rhg1_a2704_amplicon_cds		
33	rhg1_noir_amplicon_cds		
34	rhg1_lee_amplicon_cds		
35	rhg1_pi200499_amplicon_cds		
36	rhg1_A3244_amplicon_cds_2		
37	rhg1_peking_amplicon_cds_2		
38	rhg1_toyosuzu_amplicon_cds_2		
39	rhg1_will_amplicon_cds_2		
40	rhg1_a2704_amplicon_cds_2		
41	rhg1_noir_amplicon_cds_2		
42	rhg1_lee_amplicon_cds_2		
43	rhg1_pi200499_amplicon_cds_2		

Seq Num	Seq ID	Coding Sequence	AA No.
44	rhg4_a3244_amplicon	79-2242,2958-3478	1116
45	rhg4_Minsoy_amplicon	79-2242,2958-3478	1117
46	rhg4_Jack_amplicon	79-2242,2958-3478	1118
47	rhg4_peking_amplicon	79-2242,2958-3478	1119

Seq Num	Seq ID	Primer location on 318O13_region_A3	
48	318O13_region_A3_forward	111727-111756	
49	318O13_region_A3_reverse	115206-115177	

Seq Num	Seq ID		
50	rhg4_A3244_amplicon_cds		
51	rhg4_Minsoy_amplicon_cds		
52	rhg4_Jack_amplicon_cds		
53	rhg4_peking_amplicon_cds		

Seq Num	Seq ID		
54	240O17_region_G3__289711_11		
55	240O17_region_G3__236585_14		
56	240O17_region_G3__168772_13		
57	240O17_region_G3__332420_21		
58	240O17_region_G3__228126_18		
59	240O17_region_G3__139723_11		
60	240O17_region_G3__280585_14		
61	240O17_region_G3__70509_14		

Seq Num	Seq ID		
62	240O17_region_G3__50537_17		
63	240O17_region_G3__231556_17		
64	240O17_region_G3__117057_11		
65	240O17_region_G3__23092_13		
66	240O17_region_G3__297741_14		
67	240O17_region_G3__206502_14		
68	240O17_region_G3__221223_13		
69	240O17_region_G3__169084_14		
70	240O17_region_G3__94891_14		
71	240O17_region_G3__281852_61		
72	240O17_region_G3__46583_12		
73	240O17_region_G3__306835_13		
74	240O17_region_G3__85471_12		
75	240O17_region_G3__257208_12		
76	240O17_region_G3__150390_17		
77	240O17_region_G3__34697_75		
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79	240O17_region_G3__40513_22		
80	240O17_region_G3__268602_14		
81	240O17_region_G3__25357_13		
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83	240O17_region_G3__139131_13		
84	240O17_region_G3__203855_12		
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86	240O17_region_G3__320907_12		
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88	240O17_region_G3__206516_17		
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91	240O17_region_G3__187129_12		
92	240O17_region_G3__214106_13		
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308	318O13_region_A3__448857_15		
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311	318O13_region_A3__382583_13		

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343	318O13_region_A3__101255_15		
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352	515O02_region_G2__72308_77		
353	515O02_region_G2__99475_19		
354	515O02_region_G2__118615_18		
355	515O02_region_G2__119001_46		
356	515O02_region_G2__118958_43		
357	515O02_region_G2__17197_13		
358	515O02_region_G2__105163_29		
359	515O02_region_G2__111335_13		
360	515O02_region_G2__106396_13		
361	515O02_region_G2__59229_17		

Seq Num	Seq ID		
362	515O02_region_G2__73795_20		
363	515O02_region_G2__85664_20		
364	515O02_region_G2__36921_17		
365	515O02_region_G2__124150_19		
366	515O02_region_G2__5089_14		
367	515O02_region_G2__58221_15		
368	515O02_region_G2__96139_14		
369	515O02_region_G2__70595_13		
370	515O02_region_G2__4340_15		
371	515O02_region_G2__90417_11		
372	515O02_region_G2__49711_17		
373	515O02_region_G2__63053_13		
374	515O02_region_G2__63076_14		
375	515O02_region_G2__44442_12		
376	515O02_region_G2__44422_19		
377	515O02_region_G2__44158_19		
378	515O02_region_G2__44141_17		
379	515O02_region_G2__90762_17		
380	515O02_region_G2__106241_14		
381	515O02_region_G2__109676_12		
382	515O02_region_G2__86242_14		
383	515O02_region_G2__83109_12		
384	515O02_region_G2__10461_15		
385	515O02_region_G2__67608_15		
386	515O02_region_G2__63275_46		
387	515O02_region_G2__62405_14		
388	515O02_region_G2__33563_12		
389	515O02_region_G2__33146_14		
390	515O02_region_G2__102179_29		
391	515O02_region_G2__2646_15		
392	515O02_region_G2__76652_24		
393	515O02_region_G2__66280_14		
394	515O02_region_G2__54768_13		
395	515O02_region_G2__62580_14		
396	515O02_region_G2__34598_55		
397	515O02_region_G2__77680_13		
398	515O02_region_G2__77693_12		
399	515O02_region_G2__97392_14		
400	515O02_region_G2__97359_15		

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
401	240O17_region_G3__289711_11_Forward_Primer	289637	289661
402	240O17_region_G3__289711_11_Reverse_Primer	289756	289732
403	240O17_region_G3__236585_14_Forward_Primer	236511	236535
404	240O17_region_G3__236585_14_Reverse_Primer	236638	236614
405	240O17_region_G3__168772_13_Forward_Primer	168683	168707
406	240O17_region_G3__168772_13_Reverse_Primer	168811	168786
407	240O17_region_G3__332420_21_Forward_Primer	332375	332399
408	240O17_region_G3__332420_21_Reverse_Primer	332505	332481

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Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
409	240O17_region_G3__228126_18_Forward_Primer	228048	228072
410	240O17_region_G3__228126_18_Reverse_Primer	228182	228158
411	240O17_region_G3__139723_11_Forward_Primer	139666	139690
412	240O17_region_G3__139723_11_Reverse_Primer	139802	139778
413	240O17_region_G3__280585_14_Forward_Primer	280524	280550
414	240O17_region_G3__280585_14_Reverse_Primer	280661	280637
415	240O17_region_G3__70509_14_Forward_Primer	70478	70502
416	240O17_region_G3__70509_14_Reverse_Primer	70616	70592
417	240O17_region_G3__50537_17_Forward_Primer	50455	50479
418	240O17_region_G3__50537_17_Reverse_Primer	50593	50569
419	240O17_region_G3__231556_17_Forward_Primer	231468	231492
420	240O17_region_G3__231556_17_Reverse_Primer	231606	231582
421	240O17_region_G3__117057_11_Forward_Primer	117029	117053
422	240O17_region_G3__117057_11_Reverse_Primer	117169	117145
423	240O17_region_G3__23092_13_Forward_Primer	23010	23034
424	240O17_region_G3__23092_13_Reverse_Primer	23151	23127
425	240O17_region_G3__297741_14_Forward_Primer	297680	297704
426	240O17_region_G3__297741_14_Reverse_Primer	297823	297799
427	240O17_region_G3__206502_14_Forward_Primer	206456	206480
428	240O17_region_G3__206502_14_Reverse_Primer	206600	206581
429	240O17_region_G3__221223_13_Forward_Primer	221134	221158
430	240O17_region_G3__221223_13_Reverse_Primer	221278	221254
431	240O17_region_G3__169084_14_Forward_Primer	169051	169075
432	240O17_region_G3__169084_14_Reverse_Primer	169196	169173
433	240O17_region_G3__94891_14_Forward_Primer	94784	94808
434	240O17_region_G3__94891_14_Reverse_Primer	94929	94905
435	240O17_region_G3__7439_12_Forward_Primer	7397	7421
436	240O17_region_G3__7439_12_Reverse_Primer	7542	7518
437	240O17_region_G3__281852_61_Forward_Primer	281797	281821
438	240O17_region_G3__281852_61_Reverse_Primer	281943	281919
439	240O17_region_G3__46583_12_Forward_Primer	46554	46578
440	240O17_region_G3__46583_12_Reverse_Primer	46700	46676
441	240O17_region_G3__306835_13_Forward_Primer	306727	306751
442	240O17_region_G3__306835_13_Reverse_Primer	306874	306849
443	240O17_region_G3__85471_12_Forward_Primer	85359	85383
444	240O17_region_G3__85471_12_Reverse_Primer	85507	85483
445	240O17_region_G3__257208_12_Forward_Primer	257129	257153
446	240O17_region_G3__257208_12_Reverse_Primer	257278	257254
447	240O17_region_G3__150390_17_Forward_Primer	150327	150351
448	240O17_region_G3__150390_17_Reverse_Primer	150476	150452
449	240O17_region_G3__34697_75_Forward_Primer	34662	34685
450	240O17_region_G3__34697_75_Reverse_Primer	34811	34787
451	240O17_region_G3__150374_13_Forward_Primer	150327	150351
452	240O17_region_G3__150374_13_Reverse_Primer	150476	150452
453	240O17_region_G3__40513_22_Forward_Primer	40422	40446
454	240O17_region_G3__40513_22_Reverse_Primer	40572	40548
455	240O17_region_G3__268602_14_Forward_Primer	268555	268579
456	240O17_region_G3__268602_14_Reverse_Primer	268705	268681
457	240O17_region_G3__25357_13_Forward_Primer	25271	25295

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
458	240O17_region_G3__25357_13_Reverse_Primer	25422	25402
459	240O17_region_G3__137548_13_Forward_Primer	139088	139111
459	240O17_region_G3__137548_13_Forward_Primer	137505	137528
460	240O17_region_G3__137548_13_Reverse_Primer	139239	139215
460	240O17_region_G3__137548_13_Reverse_Primer	137656	137632
461	240O17_region_G3__139131_13_Forward_Primer	139088	139111
462	240O17_region_G3__139131_13_Reverse_Primer	139239	139215
463	240O17_region_G3__203855_12_Forward_Primer	203749	203773
464	240O17_region_G3__203855_12_Reverse_Primer	203901	203877
465	240O17_region_G3__199049_15_Forward_Primer	199008	199033
466	240O17_region_G3__199049_15_Reverse_Primer	199160	199136
467	240O17_region_G3__320907_12_Forward_Primer	320885	320906
468	240O17_region_G3__320907_12_Reverse_Primer	321038	321015
469	240O17_region_G3__16407_17_Forward_Primer	16330	16354
470	240O17_region_G3__16407_17_Reverse_Primer	16483	16459
471	240O17_region_G3__206516_17_Forward_Primer	206482	206506
472	240O17_region_G3__206516_17_Reverse_Primer	206635	206616
473	240O17_region_G3__264495_13_Forward_Primer	264423	264447
474	240O17_region_G3__264495_13_Reverse_Primer	264577	264553
475	240O17_region_G3__156785_13_Forward_Primer	156713	156737
476	240O17_region_G3__156785_13_Reverse_Primer	156868	156844
477	240O17_region_G3__187129_12_Forward_Primer	187068	187092
478	240O17_region_G3__187129_12_Reverse_Primer	187223	187199
479	240O17_region_G3__214106_13_Forward_Primer	214042	214067
480	240O17_region_G3__214106_13_Reverse_Primer	214197	214173
481	240O17_region_G3__149013_12_Forward_Primer	148898	148922
482	240O17_region_G3__149013_12_Reverse_Primer	149053	149027
483	240O17_region_G3__326352_16_Forward_Primer	326311	326335
484	240O17_region_G3__326352_16_Reverse_Primer	326467	326443
485	240O17_region_G3__278962_12_Forward_Primer	278933	278957
486	240O17_region_G3__278962_12_Reverse_Primer	279089	279065
487	240O17_region_G3__256930_13_Forward_Primer	256850	256874
488	240O17_region_G3__256930_13_Reverse_Primer	257006	256982
489	240O17_region_G3__29646_14_Forward_Primer	29589	29613
490	240O17_region_G3__29646_14_Reverse_Primer	29746	29721
491	240O17_region_G3__29618_13_Forward_Primer	29589	29613
492	240O17_region_G3__29618_13_Reverse_Primer	29746	29721
493	240O17_region_G3__108561_14_Forward_Primer	108518	108542
494	240O17_region_G3__108561_14_Reverse_Primer	108675	108651
495	240O17_region_G3__143975_14_Forward_Primer	143939	143964
496	240O17_region_G3__143975_14_Reverse_Primer	144096	144072
497	240O17_region_G3__108431_20_Forward_Primer	108362	108386
498	240O17_region_G3__108431_20_Reverse_Primer	108520	108497
499	240O17_region_G3__281764_11_Forward_Primer	281645	281669
500	240O17_region_G3__281764_11_Reverse_Primer	281803	281779
501	240O17_region_G3__130058_15_Forward_Primer	129994	130018
502	240O17_region_G3__130058_15_Reverse_Primer	130153	130129
503	240O17_region_G3__310590_52_Forward_Primer	310533	310557
504	240O17_region_G3__310590_52_Reverse_Primer	310692	310668

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
505	240O17_region_G3__313405_14_Forward_Primer	313345	313369
506	240O17_region_G3__313405_14_Reverse_Primer	313505	313481
507	240O17_region_G3__302190_13_Forward_Primer	302093	302119
508	240O17_region_G3__302190_13_Reverse_Primer	302253	302229
509	240O17_region_G3__225343_17_Forward_Primer	225315	225338
510	240O17_region_G3__225343_17_Reverse_Primer	225475	225451
511	240O17_region_G3__208823_14_Forward_Primer	208760	208784
512	240O17_region_G3__208823_14_Reverse_Primer	208921	208897
513	240O17_region_G3__74285_11_Forward_Primer	74220	74244
514	240O17_region_G3__74285_11_Reverse_Primer	74382	74358
515	240O17_region_G3__109052_16_Forward_Primer	108999	109023
516	240O17_region_G3__109052_16_Reverse_Primer	109161	109137
517	240O17_region_G3__6395_12_Forward_Primer	6285	6309
518	240O17_region_G3__6395_12_Reverse_Primer	6447	6423
519	240O17_region_G3__244905_16_Forward_Primer	244865	244890
520	240O17_region_G3__244905_16_Reverse_Primer	245028	245004
521	240O17_region_G3__244956_13_Forward_Primer	244865	244890
522	240O17_region_G3__244956_13_Reverse_Primer	245028	245004
523	240O17_region_G3__117220_13_Forward_Primer	117175	117199
524	240O17_region_G3__117220_13_Reverse_Primer	117339	117315
525	240O17_region_G3__134707_14_Forward_Primer	134584	134608
526	240O17_region_G3__134707_14_Reverse_Primer	134749	134725
527	240O17_region_G3__35078_13_Forward_Primer	34990	35013
528	240O17_region_G3__35078_13_Reverse_Primer	35157	35133
529	240O17_region_G3__210506_16_Forward_Primer	210477	210501
530	240O17_region_G3__210506_16_Reverse_Primer	210644	210620
531	240O17_region_G3__116961_26_Forward_Primer	116885	116909
532	240O17_region_G3__116961_26_Reverse_Primer	117053	117029
533	240O17_region_G3__51073_13_Forward_Primer	50979	51003
534	240O17_region_G3__51073_13_Reverse_Primer	51147	51123
535	240O17_region_G3__55291_15_Forward_Primer	55164	55188
536	240O17_region_G3__55291_15_Reverse_Primer	55333	55309
537	240O17_region_G3__229651_18_Forward_Primer	229615	229639
538	240O17_region_G3__229651_18_Reverse_Primer	229784	229760
539	240O17_region_G3__303308_19_Forward_Primer	303284	303307
540	240O17_region_G3__303308_19_Reverse_Primer	303454	303429
541	240O17_region_G3__168373_20_Forward_Primer	168262	168286
542	240O17_region_G3__168373_20_Reverse_Primer	168432	168408
543	240O17_region_G3__253333_17_Forward_Primer	253257	253281
544	240O17_region_G3__253333_17_Reverse_Primer	253428	253404
545	240O17_region_G3__5791_13_Forward_Primer	5766	5790
546	240O17_region_G3__5791_13_Reverse_Primer	5937	5912
547	240O17_region_G3__206841_19_Forward_Primer	206821	206840
548	240O17_region_G3__206841_19_Reverse_Primer	206993	206969
549	240O17_region_G3__202827_12_Forward_Primer	202782	202806
550	240O17_region_G3__202827_12_Reverse_Primer	202956	202932
551	240O17_region_G3__322656_13_Forward_Primer	322572	322598
552	240O17_region_G3__322656_13_Reverse_Primer	322748	322724
553	240O17_region_G3__111841_14_Forward_Primer	111709	111733

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
554	240O17_region_G3__111841_14_Reverse_Primer	111886	111861
555	240O17_region_G3__192719_13_Forward_Primer	192589	192613
556	240O17_region_G3__192719_13_Reverse_Primer	192767	192743
557	240O17_region_G3__195630_17_Forward_Primer	195490	195514
558	240O17_region_G3__195630_17_Reverse_Primer	195672	195648
559	240O17_region_G3__69999_13_Forward_Primer	69858	69881
560	240O17_region_G3__69999_13_Reverse_Primer	70040	70016
561	240O17_region_G3__11176_13_Forward_Primer	11060	11084
562	240O17_region_G3__11176_13_Reverse_Primer	11243	11219
563	240O17_region_G3__228643_13_Forward_Primer	228529	228553
564	240O17_region_G3__228643_13_Reverse_Primer	228713	228689
565	240O17_region_G3__88478_19_Forward_Primer	88378	88402
566	240O17_region_G3__88478_19_Reverse_Primer	88562	88538
567	240O17_region_G3__108950_13_Forward_Primer	108838	108858
568	240O17_region_G3__108950_13_Reverse_Primer	109023	108998
569	240O17_region_G3__121054_14_Forward_Primer	120911	120935
570	240O17_region_G3__121054_14_Reverse_Primer	121096	121072
571	240O17_region_G3__188337_14_Forward_Primer	188204	188228
572	240O17_region_G3__188337_14_Reverse_Primer	191544	191520
572	240O17_region_G3__188337_14_Reverse_Primer	188391	188367
573	240O17_region_G3__255944_21_Forward_Primer	255879	255903
574	240O17_region_G3__255944_21_Reverse_Primer	256068	256044
575	240O17_region_G3__219518_14_Forward_Primer	219420	219444
576	240O17_region_G3__219518_14_Reverse_Primer	219609	219585
577	240O17_region_G3__235601_15_Forward_Primer	235483	235507
578	240O17_region_G3__235601_15_Reverse_Primer	235673	235649
579	240O17_region_G3__301529_13_Forward_Primer	301498	301522
580	240O17_region_G3__301529_13_Reverse_Primer	301689	301665
581	240O17_region_G3__94795_14_Forward_Primer	94735	94756
582	240O17_region_G3__94795_14_Reverse_Primer	94929	94905
583	240O17_region_G3__46703_23_Forward_Primer	46676	46700
584	240O17_region_G3__46703_23_Reverse_Primer	46870	46846
585	240O17_region_G3__59616_14_Forward_Primer	59539	59563
586	240O17_region_G3__59616_14_Reverse_Primer	59738	59714
587	240O17_region_G3__296933_15_Forward_Primer	296908	296932
588	240O17_region_G3__296933_15_Reverse_Primer	297113	297089
589	240O17_region_G3__192428_17_Forward_Primer	192402	192426
590	240O17_region_G3__192428_17_Reverse_Primer	192613	192589
591	240O17_region_G3__191490_14_Forward_Primer	191332	191356
592	240O17_region_G3__191490_14_Reverse_Primer	191544	191520
593	240O17_region_G3__201115_11_Forward_Primer	200994	201018
594	240O17_region_G3__201115_11_Reverse_Primer	201214	201189
595	240O17_region_G3__72882_15_Forward_Primer	72848	72874
596	240O17_region_G3__72882_15_Reverse_Primer	73068	73042
597	240O17_region_G3__69514_13_Forward_Primer	69411	69437
598	240O17_region_G3__69514_13_Reverse_Primer	69632	69608
599	240O17_region_G3__37699_47_Forward_Primer	37601	37625
600	240O17_region_G3__37699_47_Reverse_Primer	37827	37802
601	240O17_region_G3__11301_29_Forward_Primer	11274	11300

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
602	240O17_region_G3__11301_29_Reverse_Primer	11501	11477
603	240O17_region_G3__141875_12_Forward_Primer	141729	141750
604	240O17_region_G3__141875_12_Reverse_Primer	141964	141939
605	240O17_region_G3__98090_18_Forward_Primer	98037	98062
606	240O17_region_G3__98090_18_Reverse_Primer	98274	98250
607	240O17_region_G3__43298_35_Forward_Primer	43144	43168
608	240O17_region_G3__43298_35_Reverse_Primer	43387	43363
609	240O17_region_G3__262094_11_Forward_Primer	261989	262014
610	240O17_region_G3__262094_11_Reverse_Primer	262236	262211
611	240O17_region_G3__262079_15_Forward_Primer	261989	262014
612	240O17_region_G3__262079_15_Reverse_Primer	262236	262211
613	240O17_region_G3__59090_12_Forward_Primer	58986	59012
614	240O17_region_G3__59090_12_Reverse_Primer	59248	59224
615	240O17_region_G3__245723_13_Forward_Primer	245502	245526
616	240O17_region_G3__245723_13_Reverse_Primer	245766	245742
617	240O17_region_G3__194628_54_Forward_Primer	194581	194607
618	240O17_region_G3__194628_54_Reverse_Primer	194846	194822
619	240O17_region_G3__4566_16_Forward_Primer	4455	4479
620	240O17_region_G3__4566_16_Reverse_Primer	4722	4696
621	240O17_region_G3__96209_14_Forward_Primer	96119	96143
622	240O17_region_G3__96209_14_Reverse_Primer	96392	96368
623	240O17_region_G3__248715_17_Forward_Primer	248633	248657
624	240O17_region_G3__248715_17_Reverse_Primer	248906	248882
625	240O17_region_G3__71410_40_Forward_Primer	71357	71379
626	240O17_region_G3__71410_40_Reverse_Primer	71636	71611
627	240O17_region_G3__226519_13_Forward_Primer	226315	226339
628	240O17_region_G3__226519_13_Reverse_Primer	226598	226574
629	240O17_region_G3__11282_19_Forward_Primer	11217	11242
630	240O17_region_G3__11282_19_Reverse_Primer	11501	11477
631	240O17_region_G3__170504_12_Forward_Primer	170409	170433
632	240O17_region_G3__170504_12_Reverse_Primer	170694	170671
633	240O17_region_G3__40864_14_Forward_Primer	40652	40678
634	240O17_region_G3__40864_14_Reverse_Primer	40938	40912
635	240O17_region_G3__13529_14_Forward_Primer	13332	13356
636	240O17_region_G3__13529_14_Reverse_Primer	13622	13598
637	240O17_region_G3__22858_14_Forward_Primer	22675	22699
638	240O17_region_G3__22858_14_Reverse_Primer	22966	22942
639	240O17_region_G3__309211_13_Forward_Primer	309092	309118
640	240O17_region_G3__309211_13_Reverse_Primer	309383	309358
641	240O17_region_G3__55568_26_Forward_Primer	55375	55399
642	240O17_region_G3__55568_26_Reverse_Primer	55667	55642
643	240O17_region_G3__73238_16_Forward_Primer	73043	73069
644	240O17_region_G3__73238_16_Reverse_Primer	73342	73318
645	240O17_region_G3__52488_19_Forward_Primer	52413	52437
646	240O17_region_G3__52488_19_Reverse_Primer	52712	52688
647	318O13_region_A3__471518_14_Forward_Primer_Seq	471464	471488
648	318O13_region_A3__471518_14_Reverse_Primer_Seq	471567	471541
649	318O13_region_A3__231599_23_Forward_Primer_Seq	231568	231592
650	318O13_region_A3__231599_23_Reverse_Primer_Seq	231672	231651

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
651	318O13_region_A3__375912_13_Forward_Primer_Seq	375845	375865
652	318O13_region_A3__375912_13_Reverse_Primer_Seq	375954	375932
653	318O13_region_A3__180013_12_Forward_Primer_Seq	179951	179974
654	318O13_region_A3__180013_12_Reverse_Primer_Seq	180060	180038
655	318O13_region_A3__171606_14_Forward_Primer_Seq	171545	171569
656	318O13_region_A3__171606_14_Reverse_Primer_Seq	171657	171633
657	318O13_region_A3__416256_13_Forward_Primer_Seq	416180	416203
658	318O13_region_A3__416256_13_Reverse_Primer_Seq	416293	416269
659	318O13_region_A3__231395_15_Forward_Primer_Seq	231339	231363
660	318O13_region_A3__231395_15_Reverse_Primer_Seq	231461	231438
661	318O13_region_A3__5502_47Forward_Primer_Seq	5461	5485
662	318O13_region_A3__5502_47_Reverse_Primer_Seq	5585	5561
663	318O13_region_A3__93061_14_Forward_Primer_Seq	92988	93012
664	318O13_region_A3__93061_14_Reverse_Primer_Seq	93112	93090
665	318O13_region_A3__111684_19_Forward_Primer_Seq	111646	111670
666	318O13_region_A3__111684_19_Reverse_Primer_Seq	111772	111748
667	318O13_region_A3__69328_14_Forward_Primer_Seq	69246	69269
668	318O13_region_A3__69328_14_Reverse_Primer_Seq	69373	69349
669	318O13_region_A3__36529_17_Forward_Primer_Seq	36488	36512
670	318O13_region_A3__36529_17_Reverse_Primer_Seq	36617	36593
671	318O13_region_A3__139128_12_Forward_Primer_Seq	139043	139067
672	318O13_region_A3__139128_12_Reverse_Primer_Seq	139174	139150
673	318O13_region_A3__495674_13_Forward_Primer_Seq	495592	495616
674	318O13_region_A3__495674_13_Reverse_Primer_Seq	495723	495699
675	318O13_region_A3__187577_13_Forward_Primer_Seq	187482	187506
676	318O13_region_A3__187577_13_Reverse_Primer_Seq	187613	187590
677	318O13_region_A3__453036_14_Forward_Primer_Seq	452999	453023
678	318O13_region_A3__453036_14_Reverse_Primer_Seq	453132	453108
679	318O13_region_A3__374041_13_Forward_Primer_Seq	373964	373988
680	318O13_region_A3__374041_13_Reverse_Primer_Seq	374097	374073
681	318O13_region_A3__3412_11__Forward_Primer_Seq	3319	3341
682	318O13_region_A3__3412_11_Reverse_Primer_Seq	3454	3430
683	318O13_region_A3__276495_28_Forward_Primer_Seq	276462	276485
684	318O13_region_A3__276495_28_Reverse_Primer_Seq	276598	276574
685	318O13_region_A3__151839_17_Forward_Primer_Seq	151744	151768
686	318O13_region_A3__151839_17_Reverse_Primer_Seq	151882	151858
687	318O13_region_A3__292912_12_Forward_Primer_Seq	292875	292899
688	318O13_region_A3__292912_12_Reverse_Primer_Seq	293014	292990
689	318O13_region_A3__104560_12_Forward_Primer_Seq	104464	104488
690	318O13_region_A3__104560_12_Reverse_Primer_Seq	104604	104580
691	318O13_region_A3__65193_11__Forward_Primer_Seq	65155	65179
692	318O13_region_A3__65193_11_Reverse_Primer_Seq	65295	65271
693	318O13_region_A3__110573_70_Forward_Primer_Seq	110533	110559
694	318O13_region_A3__110573_70_Reverse_Primer_Seq	110674	110648
695	318O13_region_A3__65117_12_Forward_Primer_Seq	65034	65058
696	318O13_region_A3__65117_12_Reverse_Primer_Seq	65177	65153
697	318O13_region_A3__490837_16_Forward_Primer_Seq	490762	490786
698	318O13_region_A3__490837_16_Reverse_Primer_Seq	490905	490881
699	318O13_region_A3__107448_11_Forward_Primer_Seq	107385	107411

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
700	318O13_region_A3__107448_11_Reverse_Primer_Seq	107529	107505
701	318O13_region_A3__331_23_Forward_Primer_Seq	276	301
702	318O13_region_A3__331_23_Reverse_Primer_Seq	421	397
703	318O13_region_A3__193470_13_Forward_Primer_Seq	193444	193468
704	318O13_region_A3__193470_13_Reverse_Primer_Seq	193589	193565
705	318O13_region_A3__183305_14_Forward_Primer_Seq	183239	183263
706	318O13_region_A3__183305_14_Reverse_Primer_Seq	183384	183360
707	318O13_region_A3__55050_14_Forward_Primer_Seq	54998	55022
708	318O13_region_A3__55050_14_Reverse_Primer_Seq	55144	55120
709	318O13_region_A3__224693_21_Forward_Primer_Seq	224656	224682
710	318O13_region_A3__224693_21_Reverse_Primer_Seq	224803	224779
711	318O13_region_A3__207216_12_Forward_Primer_Seq	207152	207176
712	318O13_region_A3__207216_12_Reverse_Primer_Seq	207299	207276
713	318O13_region_A3__4654_22_Forward_Primer_Seq	4612	4636
714	318O13_region_A3__4654_22_Reverse_Primer_Seq	4760	4736
715	318O13_region_A3__408959_13_Forward_Primer_Seq	408918	408942
716	318O13_region_A3__408959_13_Reverse_Primer_Seq	409066	409042
717	318O13_region_A3__132288_22_Forward_Primer_Seq	132192	132216
718	318O13_region_A3__132288_22_Reverse_Primer_Seq	132340	132316
719	318O13_region_A3__292822_20_Forward_Primer_Seq	292747	292771
720	318O13_region_A3__292822_20_Reverse_Primer_Seq	292895	292871
721	318O13_region_A3__311076_12_Forward_Primer_Seq	311027	311051
722	318O13_region_A3__311076_12_Reverse_Primer_Seq	311175	311152
723	318O13_region_A3__509623_13_Forward_Primer_Seq	509584	509608
724	318O13_region_A3__509623_13_Reverse_Primer_Seq	509732	509708
725	318O13_region_A3__190404_14_Forward_Primer_Seq	190358	190382
726	318O13_region_A3__190404_14_Reverse_Primer_Seq	190506	190482
727	318O13_region_A3__164916_15_Forward_Primer_Seq	164808	164832
728	318O13_region_A3__164916_15_Reverse_Primer_Seq	164957	164933
729	318O13_region_A3__21028_13_Forward_Primer_Seq	21001	21026
730	318O13_region_A3__21028_13_Reverse_Primer_Seq	21150	21126
731	318O13_region_A3__208012_17_Forward_Primer_Seq	207955	207979
732	318O13_region_A3__208012_17_Reverse_Primer_Seq	208104	208085
733	318O13_region_A3__484089_14_Forward_Primer_Seq	484036	484060
734	318O13_region_A3__484089_14_Reverse_Primer_Seq	484185	484161
735	318O13_region_A3__332780_17_Forward_Primer_Seq	332723	332747
736	318O13_region_A3__332780_17_Reverse_Primer_Seq	332872	332853
737	318O13_region_A3__480137_37_Forward_Primer_Seq	480059	480084
738	318O13_region_A3__480137_37_Reverse_Primer_Seq	480208	480182
739	318O13_region_A3__441056_14_Forward_Primer_Seq	441011	441035
740	318O13_region_A3__441056_14_Reverse_Primer_Seq	441161	441138
741	318O13_region_A3__77486_11_Forward_Primer_Seq	77447	77471
742	318O13_region_A3__77486_11_Reverse_Primer_Seq	77597	77573
743	318O13_region_A3__272468_11_Forward_Primer_Seq	272423	272447
744	318O13_region_A3__272468_11_Reverse_Primer_Seq	272573	272549
745	318O13_region_A3__425319_17_Forward_Primer_Seq	425233	425257
746	318O13_region_A3__425319_17_Reverse_Primer_Seq	425383	425359
747	318O13_region_A3__413879_31_Forward_Primer_Seq	413835	413859
748	318O13_region_A3__413879_31_Reverse_Primer_Seq	413985	413961

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
749	318O13_region_A3__80477_64_Forward_Primer_Seq	80440	80464
750	318O13_region_A3__80477_64_Reverse_Primer_Seq	80591	80567
751	318O13_region_A3__277272_50_Forward_Primer_Seq	277213	277237
752	318O13_region_A3__277272_50_Reverse_Primer_Seq	277364	277340
753	318O13_region_A3__509642_13_Forward_Primer_Seq	509604	509628
754	318O13_region_A3__509642_13_Reverse_Primer_Seq	509755	509731
755	318O13_region_A3__321771_14_Forward_Primer_Seq	321663	321687
756	318O13_region_A3__321771_14_Reverse_Primer_Seq	321815	321791
757	318O13_region_A3__26788_12_Forward_Primer_Seq	26734	26758
758	318O13_region_A3__26788_12_Reverse_Primer_Seq	26886	26862
759	318O13_region_A3__262706_16_Forward_Primer_Seq	262649	262673
760	318O13_region_A3__262706_16_Reverse_Primer_Seq	262802	262778
761	318O13_region_A3__243928_16_Forward_Primer_Seq	243891	243915
762	318O13_region_A3__243928_16_Reverse_Primer_Seq	244044	244020
763	318O13_region_A3__23246_148_Forward_Primer_Seq	23215	23239
764	318O13_region_A3__23246_148_Reverse_Primer_Seq	23368	23344
765	318O13_region_A3__165406_12_Forward_Primer_Seq	165367	165391
766	318O13_region_A3__165406_12_Reverse_Primer_Seq	165521	165497
767	318O13_region_A3__486294_14_Forward_Primer_Seq	486208	486232
768	318O13_region_A3__486294_14_Reverse_Primer_Seq	486362	486338
769	318O13_region_A3__46754_12_Forward_Primer_Seq	46661	46685
770	318O13_region_A3__46754_12_Reverse_Primer_Seq	46816	46792
771	318O13_region_A3__381116_15_Forward_Primer_Seq	381080	381104
772	318O13_region_A3__381116_15_Reverse_Primer_Seq	381235	381211
773	318O13_region_A3__350369_11_Forward_Primer_Seq	350295	350319
774	318O13_region_A3__350369_11_Reverse_Primer_Seq	350450	350426
775	318O13_region_A3__138841_13_Forward_Primer_Seq	138795	138819
776	318O13_region_A3__138841_13_Reverse_Primer_Seq	138950	138926
777	318O13_region_A3__12158_142_Forward_Primer_Seq	12117	12141
778	318O13_region_A3__12158_142_Reverse_Primer_Seq	12272	12248
779	318O13_region_A3__315368_13_Forward_Primer_Seq	315310	315334
780	318O13_region_A3__315368_13_Reverse_Primer_Seq	315465	315441
781	318O13_region_A3__307549_13_Forward_Primer_Seq	307464	307488
782	318O13_region_A3__307549_13_Reverse_Primer_Seq	307619	307595
783	318O13_region_A3__159857_14_Forward_Primer_Seq	159772	159796
784	318O13_region_A3__159857_14_Reverse_Primer_Seq	159928	159904
785	318O13_region_A3__140551_15_Forward_Primer_Seq	140454	140478
786	318O13_region_A3__140551_15_Reverse_Primer_Seq	140610	140586
787	318O13_region_A3__279869_11_Forward_Primer_Seq	279797	279821
788	318O13_region_A3__279869_11_Reverse_Primer_Seq	279953	279929
789	318O13_region_A3__78292_35_Forward_Primer_Seq	78265	78291
790	318O13_region_A3__78292_35_Reverse_Primer_Seq	78422	78397
791	318O13_region_A3__185019_12_Forward_Primer_Seq	184953	184977
792	318O13_region_A3__185019_12_Reverse_Primer_Seq	185111	185087
793	318O13_region_A3__409164_13_Forward_Primer_Seq	409082	409106
794	318O13_region_A3__409164_13_Reverse_Primer_Seq	409240	409219
795	318O13_region_A3__75392_14_Forward_Primer_Seq	75287	75311
796	318O13_region_A3__75392_14_Reverse_Primer_Seq	75445	75421
797	318O13_region_A3__231320_12_Forward_Primer_Seq	231269	231293

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
798	318O13_region_A3__231320_12_Reverse_Primer_Seq	231429	231405
799	318O13_region_A3__381102_14_Forward_Primer_Seq	381041	381064
800	318O13_region_A3__381102_14_Reverse_Primer_Seq	381201	381176
801	318O13_region_A3__491826_15_Forward_Primer_Seq	491753	491777
802	318O13_region_A3__491826_15_Reverse_Primer_Seq	491914	491891
803	318O13_region_A3__56365_21_Forward_Primer_Seq	56336	56360
804	318O13_region_A3__56365_21_Reverse_Primer_Seq	56497	56473
805	318O13_region_A3__372628_15_Forward_Primer_Seq	372554	372578
806	318O13_region_A3__372628_15_Reverse_Primer_Seq	372715	372691
807	318O13_region_A3__217037_11_Forward_Primer_Seq	216919	216943
808	318O13_region_A3__217037_11_Reverse_Primer_Seq	217081	217057
809	318O13_region_A3__302609_11_Forward_Primer_Seq	302575	302599
810	318O13_region_A3__302609_11_Reverse_Primer_Seq	302737	302713
811	318O13_region_A3__341804_11_Forward_Primer_Seq	341686	341710
812	318O13_region_A3__341804_11_Reverse_Primer_Seq	341848	341824
807	318O13_region_A3__217037_11_Forward_Primer_Seq	216919	216943
808	318O13_region_A3__217037_11_Reverse_Primer_Seq	217081	217057
813	318O13_region_A3__264929_68_Forward_Primer_Seq	264862	264886
814	318O13_region_A3__264929_68_Reverse_Primer_Seq	265024	265000
815	318O13_region_A3__55499_12__Forward_Primer_Seq	55400	55424
816	318O13_region_A3__55499_12_Reverse_Primer_Seq	55563	55539
817	318O13_region_A3__295634_14_Forward_Primer_Seq	295538	295562
818	318O13_region_A3__295634_14_Reverse_Primer_Seq	295702	295677
819	318O13_region_A3__269358_15_Forward_Primer_Seq	269242	269266
820	318O13_region_A3__269358_15_Reverse_Primer_Seq	269406	269382
821	318O13_region_A3__457009_24_Forward_Primer_Seq	456924	456948
822	318O13_region_A3__457009_24_Reverse_Primer_Seq	457088	457064
823	318O13_region_A3__176598_14_Forward_Primer_Seq	176554	176578
824	318O13_region_A3__176598_14_Reverse_Primer_Seq	176718	176694
825	318O13_region_A3__278266_12_Forward_Primer_Seq	278210	278234
826	318O13_region_A3__278266_12_Reverse_Primer_Seq	278376	278350
827	318O13_region_A3__391810_12_Forward_Primer_Seq	391683	391707
828	318O13_region_A3__391810_12_Reverse_Primer_Seq	391851	391826
829	318O13_region_A3__269485_15_Forward_Primer_Seq	269383	269407
830	318O13_region_A3__269485_15_Reverse_Primer_Seq	269551	269527
831	318O13_region_A3__359247_17_Forward_Primer_Seq	359218	359243
832	318O13_region_A3__359247_17_Reverse_Primer_Seq	359386	359362
833	318O13_region_A3__315094_13_Forward_Primer_Seq	314976	315002
834	318O13_region_A3__315094_13_Reverse_Primer_Seq	315145	315120
835	318O13_region_A3__307823_13_Forward_Primer_Seq	307784	307809
836	318O13_region_A3__307823_13_Reverse_Primer_Seq	307953	307927
837	318O13_region_A3__248588_15_Forward_Primer_Seq	248540	248564
838	318O13_region_A3__248588_15_Reverse_Primer_Seq	248709	248684
839	318O13_region_A3__252426_85_Forward_Primer_Seq	252398	252423
840	318O13_region_A3__252426_85_Reverse_Primer_Seq	252568	252543
841	318O13_region_A3__513314_16_Forward_Primer_Seq	513209	513233
842	318O13_region_A3__513314_16_Reverse_Primer_Seq	513379	513355
843	318O13_region_A3__68183_14_Forward_Primer_Seq	68108	68132
844	318O13_region_A3__68183_14_Reverse_Primer_Seq	68279	68255

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
845	318O13_region_A3__471191_13_Forward_Primer_Seq	471059	471083
846	318O13_region_A3__471191_13_Reverse_Primer_Seq	471231	471206
847	318O13_region_A3__163547_18_Forward_Primer_Seq	163459	163483
848	318O13_region_A3__163547_18_Reverse_Primer_Seq	163632	163608
849	318O13_region_A3__417867_15_Forward_Primer_Seq	417839	417863
850	318O13_region_A3__417867_15_Reverse_Primer_Seq	418014	417990
851	318O13_region_A3__332465_14_Forward_Primer_Seq	332346	332370
852	318O13_region_A3__332465_14_Reverse_Primer_Seq	332523	332499
853	318O13_region_A3__207697_14_Forward_Primer_Seq	207578	207602
854	318O13_region_A3__207697_14_Reverse_Primer_Seq	207755	207731
855	318O13_region_A3__277229_43_Forward_Primer_Seq	277186	277210
856	318O13_region_A3__277229_43_Reverse_Primer_Seq	277364	277340
857	318O13_region_A3__36366_11__Forward_Primer_Seq	36323	36345
858	318O13_region_A3__36366_11_Reverse_Primer_Seq	36501	36477
859	318O13_region_A3__91970_12__Forward_Primer_Seq	91938	91962
860	318O13_region_A3__91970_12_Reverse_Primer_Seq	92116	92091
861	318O13_region_A3__211533_11_Forward_Primer_Seq	211406	211430
862	318O13_region_A3__211533_11_Reverse_Primer_Seq	211585	211561
863	318O13_region_A3__336301_11_Forward_Primer_Seq	336174	336198
864	318O13_region_A3__336301_11_Reverse_Primer_Seq	336353	336329
865	318O13_region_A3__441603_14_Forward_Primer_Seq	441539	441563
866	318O13_region_A3__441603_14_Reverse_Primer_Seq	441718	441694
867	318O13_region_A3__468354_15_Forward_Primer_Seq	468263	468287
868	318O13_region_A3__468354_15_Reverse_Primer_Seq	468442	468418
869	318O13_region_A3__188983_18_Forward_Primer_Seq	188855	188879
870	318O13_region_A3__188983_18_Reverse_Primer_Seq	189035	189009
871	318O13_region_A3__115502_17_Forward_Primer_Seq	115469	115493
872	318O13_region_A3__115502_17_Reverse_Primer_Seq	115649	115625
873	318O13_region_A3__163006_13_Forward_Primer_Seq	162972	162996
874	318O13_region_A3__163006_13_Reverse_Primer_Seq	163153	163129
875	318O13_region_A3__119283_14_Forward_Primer_Seq	119199	119224
876	318O13_region_A3__119283_14_Reverse_Primer_Seq	119381	119357
877	318O13_region_A3__491126_11_Forward_Primer_Seq	491062	491086
878	318O13_region_A3__491126_11_Reverse_Primer_Seq	491244	491220
879	318O13_region_A3__99512_21__Forward_Primer_Seq	99398	99422
880	318O13_region_A3__99512_21_Reverse_Primer_Seq	99581	99557
881	318O13_region_A3__280291_17_Forward_Primer_Seq	280201	280226
882	318O13_region_A3__280291_17_Reverse_Primer_Seq	280385	280361
883	318O13_region_A3__138443_19_Forward_Primer_Seq	138304	138329
884	318O13_region_A3__138443_19_Reverse_Primer_Seq	138488	138465
885	318O13_region_A3__115973_14_Forward_Primer_Seq	115832	115856
886	318O13_region_A3__115973_14_Reverse_Primer_Seq	116016	115992
887	318O13_region_A3__329977_14_Forward_Primer_Seq	329864	329889
888	318O13_region_A3__329977_14_Reverse_Primer_Seq	330050	330026
889	318O13_region_A3__205203_14_Forward_Primer_Seq	205090	205115
890	318O13_region_A3__205203_14_Reverse_Primer_Seq	205276	205252
891	318O13_region_A3__153114_12_Forward_Primer_Seq	152969	152993
892	318O13_region_A3__153114_12_Reverse_Primer_Seq	153156	153132
893	318O13_region_A3__34581_13_Forward_Primer_Seq	34523	34547

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
894	318O13_region_A3__34581_13_Reverse_Primer_Seq	34712	34688
895	318O13_region_A3__292577_19_Forward_Primer_Seq	292549	292573
896	318O13_region_A3__292577_19_Reverse_Primer_Seq	292739	292715
897	318O13_region_A3__445391_20_Forward_Primer_Seq	445356	445382
898	318O13_region_A3__445391_20_Reverse_Primer_Seq	445547	445523
899	318O13_region_A3__350540_17_Forward_Primer_Seq	350421	350445
900	318O13_region_A3__350540_17_Reverse_Primer_Seq	350612	350588
901	318O13_region_A3__453879_15_Forward_Primer_Seq	453725	453750
902	318O13_region_A3__453879_15_Reverse_Primer_Seq	453918	453894
903	318O13_region_A3__201246_13_Forward_Primer_Seq	201128	201153
904	318O13_region_A3__201246_13_Reverse_Primer_Seq	201321	201297
905	318O13_region_A3__326020_13_Forward_Primer_Seq	325902	325927
906	318O13_region_A3__326020_13_Reverse_Primer_Seq	326095	326071
907	318O13_region_A3__503801_14_Forward_Primer_Seq	503656	503680
908	318O13_region_A3__503801_14_Reverse_Primer_Seq	503849	503823
909	318O13_region_A3__302400_52_Forward_Primer_Seq	302283	302307
910	318O13_region_A3__302400_52_Reverse_Primer_Seq	302481	302456
911	318O13_region_A3__448857_15_Forward_Primer_Seq	448748	448772
912	318O13_region_A3__448857_15_Reverse_Primer_Seq	448947	448924
913	318O13_region_A3__48364_14_Forward_Primer_Seq	48232	48256
914	318O13_region_A3__48364_14_Reverse_Primer_Seq	48435	48412
915	318O13_region_A3__251804_48_Forward_Primer_Seq	251738	251762
916	318O13_region_A3__251804_48_Reverse_Primer_Seq	251942	251918
917	318O13_region_A3__382583_13_Forward_Primer_Seq	382549	382574
918	318O13_region_A3__382583_13_Reverse_Primer_Seq	382753	382728
919	318O13_region_A3__124737_14_Forward_Primer_Seq	124641	124665
920	318O13_region_A3__124737_14_Reverse_Primer_Seq	124846	124822
921	318O13_region_A3__124766_13_Forward_Primer_Seq	124641	124665
922	318O13_region_A3__124766_13_Reverse_Primer_Seq	124846	124822
923	318O13_region_A3__461351_16_Forward_Primer_Seq	461218	461242
924	318O13_region_A3__461351_16_Reverse_Primer_Seq	461426	461402
925	318O13_region_A3__64953_19__Forward_Primer_Seq	64798	64823
926	318O13_region_A3__64953_19_Reverse_Primer_Seq	65011	64987
927	318O13_region_A3__366586_13_Forward_Primer_Seq	366508	366532
928	318O13_region_A3__366586_13_Reverse_Primer_Seq	366722	366698
929	318O13_region_A3__46190_15__Forward_Primer_Seq	46012	46037
930	318O13_region_A3__46190_15_Reverse_Primer_Seq	46228	46205
931	318O13_region_A3__81016_11__Forward_Primer_Seq	80927	80952
932	318O13_region_A3__81016_11_Reverse_Primer_Seq	81146	81122
933	318O13_region_A3__134426_14_Forward_Primer_Seq	134253	134277
934	318O13_region_A3__134426_14_Reverse_Primer_Seq	134474	134449
935	318O13_region_A3__292724_14_Forward_Primer_Seq	292549	292573
936	318O13_region_A3__292724_14_Reverse_Primer_Seq	292771	292747
937	318O13_region_A3__187096_17_Forward_Primer_Seq	187058	187082
938	318O13_region_A3__187096_17_Reverse_Primer_Seq	187282	187257
939	318O13_region_A3__381693_13_Forward_Primer_Seq	381658	381683
940	318O13_region_A3__381693_13_Reverse_Primer_Seq	381885	381863
941	318O13_region_A3__361286_33_Forward_Primer_Seq	361173	361197
942	318O13_region_A3__361286_33_Reverse_Primer_Seq	361401	361376

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
943	318O13_region_A3__482668_14_Forward_Primer_Seq	482592	482616
944	318O13_region_A3__482668_14_Reverse_Primer_Seq	482821	482796
945	318O13_region_A3__128002_12_Forward_Primer_Seq	127882	127906
946	318O13_region_A3__128002_12_Reverse_Primer_Seq	128112	128087
947	318O13_region_A3__499270_14_Forward_Primer_Seq	499184	499208
948	318O13_region_A3__499270_14_Reverse_Primer_Seq	499422	499398
949	318O13_region_A3__231650_12_Forward_Primer_Seq	231568	231592
950	318O13_region_A3__231650_12_Reverse_Primer_Seq	231809	231788
951	318O13_region_A3__199851_13_Forward_Primer_Seq	199762	199786
952	318O13_region_A3__199851_13_Reverse_Primer_Seq	200012	199988
953	318O13_region_A3__324629_13_Forward_Primer_Seq	324540	324564
954	318O13_region_A3__324629_13_Reverse_Primer_Seq	324790	324766
955	318O13_region_A3__374190_19_Forward_Primer_Seq	374129	374152
956	318O13_region_A3__374190_19_Reverse_Primer_Seq	374394	374370
957	318O13_region_A3__460603_13_Forward_Primer_Seq	460450	460474
958	318O13_region_A3__460603_13_Reverse_Primer_Seq	460715	460691
959	318O13_region_A3__108681_14_Forward_Primer_Seq	108524	108548
960	318O13_region_A3__108681_14_Reverse_Primer_Seq	108791	108768
961	318O13_region_A3__459791_47_Forward_Primer_Seq	459639	459663
962	318O13_region_A3__459791_47_Reverse_Primer_Seq	459907	459883
963	318O13_region_A3__4257_20_Forward_Primer_Seq	4172	4196
964	318O13_region_A3__4257_20__Reverse_Primer_Seq	4450	4425
965	318O13_region_A3__238810_14_Forward_Primer_Seq	238563	238589
966	318O13_region_A3__238810_14_Reverse_Primer_Seq	238850	238826
967	318O13_region_A3__245817_14_Forward_Primer_Seq	245713	245738
968	318O13_region_A3__245817_14_Reverse_Primer_Seq	246001	245977
969	318O13_region_A3__245956_14_Forward_Primer_Seq	245713	245738
970	318O13_region_A3__245956_14_Reverse_Primer_Seq	246001	245977
971	318O13_region_A3__74148_14__Forward_Primer_Seq	74050	74075
972	318O13_region_A3__74148_14_Reverse_Primer_Seq	74338	74314
973	318O13_region_A3__74089_15__Forward_Primer_Seq	74050	74075
974	318O13_region_A3__74089_15_Reverse_Primer_Seq	74338	74314
975	318O13_region_A3__241686_12_Forward_Primer_Seq	241470	241494
976	318O13_region_A3__241686_12_Reverse_Primer_Seq	241765	241741
977	318O13_region_A3__47476_12__Forward_Primer_Seq	47280	47304
978	318O13_region_A3__47476_127_Reverse_Primer_Seq	47577	47554
979	318O13_region_A3__164550_12_Forward_Primer_Seq	164323	164347
980	318O13_region_A3__164550_12_Reverse_Primer_Seq	164621	164598
981	318O13_region_A3__101255_15_Forward_Primer_Seq	101119	101144
982	318O13_region_A3__101255_15_Reverse_Primer_Seq	101418	101392
983	515O02_region_G2__16189_11_Forward_Primer	16144	16168
984	515O02_region_G2__16189_11_Reverse_Primer	16244	16220
985	515O02_region_G2__71925_13_Forward_Primer	71880	71905
986	515O02_region_G2__71925_13_Reverse_Primer	71987	71963
987	515O02_region_G2__4707_12_Forward_Primer	4660	4684
988	515O02_region_G2__4707_12Reverse_Primer	4769	4743
989	515O02_region_G2__118904_18_Forward_Primer	118847	118871
990	515O02_region_G2__118904_18_Reverse_Primer	118957	118932
991	515O02_region_G2__13655_17_Forward_Primer	13567	13592

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992	515O02_region_G2__13655_17_Reverse_Primer	13698	13673
993	515O02_region_G2__53900_13_Forward_Primer	53843	53867
994	515O02_region_G2__53900_13_Reverse_Primer	53985	53961
995	515O02_region_G2__8079_14_Forward_Primer	8023	8047
996	515O02_region_G2__8079_14_Reverse_Primer	8167	8143
997	515O02_region_G2__9969_28_Forward_Primer	9917	9941
998	515O02_region_G2__9969_28_Reverse_Primer	10062	10038
999	515O02_region_G2__72308_77_Forward_Primer	72272	72298
1000	515O02_region_G2__72308_77_Reverse_Primer	10062	10038
1001	515O02_region_G2__99475_19_Forward_Primer	99408	99433
1002	515O02_region_G2__99475_19_Reverse_Primer	99554	99530
1003	515O02_region_G2__118615_18_Forward_Primer	118512	118535
1004	515O02_region_G2__118615_18_Reverse_Primer	118658	118634
1005	515O02_region_G2__119001_46_Forward_Primer	118931	118956
1006	515O02_region_G2__119001_46_Reverse_Primer	119079	119055
1007	515O02_region_G2__118958_43_Forward_Primer	118931	118956
1008	515O02_region_G2__118958_43_Reverse_Primer	119079	119055
1009	515O02_region_G2__17197_13_Forward_Primer	17128	17152
1010	515O02_region_G2__17197_13_Reverse_Primer	17276	17252
1011	515O02_region_G2__105163_29_Forward_Primer	105068	105092
1012	515O02_region_G2__105163_29_Reverse_Primer	105217	105192
1013	515O02_region_G2__111335_13_Forward_Primer	111308	111332
1014	515O02_region_G2__111335_13_Reverse_Primer	111458	111434
1015	515O02_region_G2__106396_13_Forward_Primer	106318	106342
1016	515O02_region_G2__106396_13_Reverse_Primer	106469	106445
1017	515O02_region_G2__59229_17_Forward_Primer	59203	59227
1018	515O02_region_G2__59229_17_Reverse_Primer	59354	59330
1019	515O02_region_G2__73795_20_Forward_Primer	73769	73793
1020	515O02_region_G2__73795_20_Reverse_Primer	73921	73896
1021	515O02_region_G2__85664_20_Forward_Primer	85586	85611
1022	515O02_region_G2__85664_20_Reverse_Primer	85738	85714
1023	515O02_region_G2__36921_17_Forward_Primer	36830	36854
1024	515O02_region_G2__36921_17_Reverse_Primer	36983	36959
1025	515O02_region_G2__124150_19_Forward_Primer	124073	124096
1026	515O02_region_G2__124150_19_Reverse_Primer	124227	124203
1027	515O02_region_G2__5089_14_Forward_Primer	4999	5024
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1029	515O02_region_G2__58221_15_Forward_Primer	58197	58220
1030	515O02_region_G2__58221_15_Reverse_Primer	58354	58330
1031	515O02_region_G2__96139_14_Forward_Primer	96022	96046
1032	515O02_region_G2__96139_14_Reverse_Primer	96182	96158
1033	515O02_region_G2__70595_13_Forward_Primer	70472	70496
1034	515O02_region_G2__70595_13_Reverse_Primer	70634	70608
1035	515O02_region_G2__4340_15_Forward_Primer	4312	4337
1036	515O02_region_G2__4340_15_Reverse_Primer	4477	4454
1037	515O02_region_G2__90417_11_Forward_Primer	90335	90359
1038	515O02_region_G2__90417_11_Reverse_Primer	90503	90479
1039	515O02_region_G2__49711_17_Forward_Primer	49652	49676
1040	515O02_region_G2__49711_17_Reverse_Primer	49820	49796

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1041	515O02_region_G2__63053_13_Forward_Primer	63005	63029
1042	515O02_region_G2__63053_13_Reverse_Primer	63173	63148
1043	515O02_region_G2__63076_14_Forward_Primer	63005	63029
1044	515O02_region_G2__63076_14_Reverse_Primer	63173	63148
1045	515O02_region_G2__44442_12_Forward_Primer	44335	44359
1046	515O02_region_G2__44442_12_Reverse_Primer	44505	44481
1047	515O02_region_G2__44422_19_Forward_Primer	44335	44359
1048	515O02_region_G2__44422_19_Reverse_Primer	44505	44481
1049	515O02_region_G2__44158_19_Forward_Primer	44075	44100
1050	515O02_region_G2__44158_19_Reverse_Primer	44252	44227
1051	515O02_region_G2__44141_17_Forward_Primer	44075	44100
1052	515O02_region_G2__44141_17_Reverse_Primer	44252	44227
1053	515O02_region_G2__90762_17_Forward_Primer	90637	90663
1054	515O02_region_G2__90762_17_Reverse_Primer	90814	90790
1055	515O02_region_G2__106241_14_Forward_Primer	106160	106184
1056	515O02_region_G2__106241_14_Reverse_Primer	106341	106317
1057	515O02_region_G2__109676_12_Forward_Primer	109609	109634
1058	515O02_region_G2__109676_12_Reverse_Primer	109793	109768
1059	515O02_region_G2__86242_14_Forward_Primer	86134	86158
1060	515O02_region_G2__86242_14_Reverse_Primer	86318	86293
1061	515O02_region_G2__83109_12_Forward_Primer	83017	83041
1062	515O02_region_G2__83109_12_Reverse_Primer	83202	83178
1063	515O02_region_G2__10461_15_Forward_Primer	10418	10442
1064	515O02_region_G2__10461_15_Reverse_Primer	10609	10585
1065	515O02_region_G2__67608_15_Forward_Primer	67552	67577
1066	515O02_region_G2__67608_15_Reverse_Primer	67745	67721
1067	515O02_region_G2__63275_46_Forward_Primer	63148	63173
1068	515O02_region_G2__63275_46_Reverse_Primer	63347	63323
1069	515O02_region_G2__62405_14_Forward_Primer	62374	62399
1070	515O02_region_G2__62405_14_Reverse_Primer	62576	62552
1071	515O02_region_G2__33563_12_Forward_Primer	33460	33484
1072	515O02_region_G2__33563_12_Reverse_Primer	33670	33646
1073	515O02_region_G2__33146_14_Forward_Primer	32949	32973
1074	515O02_region_G2__33146_14_Reverse_Primer	33191	33167
1075	515O02_region_G2__102179_29_Forward_Primer	102102	102126
1076	515O02_region_G2__102179_29_Reverse_Primer	102352	102327
1077	515O02_region_G2__2646_15_Forward_Primer	2553	2577
1078	515O02_region_G2__2646_15_Reverse_Primer	2809	2784
1079	515O02_region_G2__76652_24_Forward_Primer	76567	76591
1080	515O02_region_G2__76652_24_Reverse_Primer	76835	76812
1081	515O02_region_G2__66280_14_Forward_Primer	66052	66077
1082	515O02_region_G2__66280_14_Reverse_Primer	66334	66309
1083	515O02_region_G2__54768_13_Forward_Primer	54640	54666
1084	515O02_region_G2__54768_13_Reverse_Primer	54923	54899
1085	515O02_region_G2__62580_14_Forward_Primer	62552	62576
1086	515O02_region_G2__62580_14_Reverse_Primer	62840	62816
1087	515O02_region_G2__34598_55_Forward_Primer	34473	34497
1088	515O02_region_G2__34598_55_Reverse_Primer	34765	34739
1089	515O02_region_G2__77680_13_Forward_Primer	77444	77470

Seq Num	Seq ID	location of primer on contig start	location of primer on contig end
1090	515O02_region_G2__77680_13_Reverse_Primer	77741	77716
1091	515O02_region_G2__77693_12_Forward_Primer	77444	77470
1092	515O02_region_G2__77693_12_Reverse_Primer	77741	77716
1093	515O02_region_G2__97392_14_Forward_Primer	97255	97280
1094	515O02_region_G2__97392_14_Reverse_Primer	97554	97530
1095	515O02_region_G2__97359_15_Forward_Primer	97255	97280
1096	515O02_region_G2__97359_15_Reverse_Primer	97554	97530

Seq Num	Seq ID		
1120	consensusLRR		
1121	rhg1LRR		
1122	Rhg4LRR		

Seq Num	Seq ID	Primer location on 240O17_region_G3	
1123	240O17_region_G3_forward_1_b	45046-45072	

DETAILED DESCRIPTION OF THE INVENTION

A)   *rhg*1

The present invention provides a method for the production of a soybean plant having an *rhg*1 SCN resistant allele comprising: (A) crossing a first soybean plant having an *rhg*1 SCN resistant allele with a second soybean plant having an *rhg*1 SCN sensitive allele to produce a segregating population; (B) screening the segregating population for a member having an *rhg*1 SCN resistant allele with a first nucleic acid molecule capable of specifically hybridizing to linkage group G, wherein the first nucleic acid molecule specifically hybridizes to a second nucleic acid molecule that is linked to the *rhg*1 SCN resistant allele; and, (C) selecting the member for further crossing and selection.

15   *rhg*1 is located on linkage group G (Concibido *et al.*, *Crop Sci. 36*:1643-1650 (1996)). SCN resistant alleles of *rhg*1 provide partial resistance to SCN races 1, 2, 3, 5, 6, and 14 (Concibido *et al.* (*Crop Sci. 37*:258-264 (1997)). Also, Webb (U.S. Patent 5,491,081) reports that a QTL on linkage group G (*rhg*1) provides partial resistance to SCN races 1, 2, 3, 5, and 14. *rhg*1 and *Rhg*4 provide complete or nearly complete resistance to SCN race 3 (U.S. Patent

5,491,081). While initially thought to be a recessive gene, *rhg*1 classification as a recessive gene has been questioned.

Using bioinformatic approaches, the *rhg*1 coding region is predicted to contain either four exons (*rhg*1, *v.1*)(coding coordinates 45163-45314, 45450-45509, 46941-48763, and 48975-49573 of SEQ ID NO: 2) or two exons (*rhg1, v.2*) (coding coordinates 46798-48763 and 48975-49573 of SEQ ID NO: 3). *rhg*1, *v.1* encodes an 877 amino acid polypeptide. *rhg*1, *v.2* encodes an 854 amino acid length polypeptide. *rhg*1 codes for a Xa21-like receptor kinase (SEQ ID NOs: 1097, 1098, and 1100-1115) (Song, *et al.,. Science* 270, 1804-1806 (1995)). *rhg*1 has an extracellular leucine rich repeat (LRR) domain (*rhg*1, *v.1*, SEQ ID NO: 1097, residues 164-457; *rhg*1, *v.2*, SEQ ID NO: 1098, residues 141-434), a transmembrane domain (*rhg*1, *v.1*, SEQ ID NO: 1097, residues 508-530; *rhg*1, *v.2*, SEQ ID NO: 1098, residues 33-51, and 485-507) and serine/threonine protein kinase (STK) domain (*rhg*1, *v.1*, SEQ ID NO: 1097, residues 578-869; *rhg*1, *v.2*, SEQ ID NO: 1098, residues 555-846). In a preferred embodiment, the LRR domain has multiple LRR repeats. In a more preferred embodiment, the LRR domain has 12 LRR repeats.

To identify proteins similar to the proteins encoded by *rhg*1 candidates, database searches are performed using the predicted peptide sequences. The *rhg*1 candidate shows similarity to CAA18124, which is the *Arabidopsis* putative receptor kinase (58.4% similarity and 35.8% identity, (CLUSTALW (default parameters), Thompson *et al.*, Nucleic Acids Res. 22:4673-4680 (1994)), GCG package, Genetics Computer Group, Madison, Wisconsin), and the apple leucine-rich receptor-like protein kinase (g3641252) (53.2% similarity and 31.5% identity, (CLUSTALW (default parameters))), which has both LLR and STK domains, showing conservation in both the LLR and STK domains. The predicted LRR extracellular domain shows similarity to the tomato resistance genes *Cf-2.1* (*Lycopersicon pimpinellifolium*) (66.9% similarity and 45.4% identity (CLUSTALW (default parameters))) and *Cf-2.2* (*Lycopersicon pimpinellifolium*) (66.9% similarity and 45.4% identity (CLUSTALW (default parameters))).

Figure 1 is an alignment of the LRR domain of the *rhg*1 gene. A consensus sequence for the LRR is shown as the top row of the alignment. Each row of amino acids represents an LRR domain. The boxed region indicates the putative β-turn/ β-sheet structural motif postulated to be involved in ligand binding (Jones and Jones, *Adv. Bot. Res. Incorp. Adv. Plant Path.* 24;89-167

5   (1997)). The hydrophobic leucine residues are thought to project into the core of the protein while the flanking amino acids are thought to be solvent exposed where they may interact with the ligand (Kobe and Deisenhofer, *Nature* 374; 183-186 (1995)). Non-conservative changes in this region are thought to affect folding. An "x" represents an arbitrary amino acid while an "a" represents a hydrophobic residue (leucine, isoleucine, methionine, valine, or phenylalanine).

10   Amino acid substitutions between resistant and sensitive phenotypes are bordered by a double line. The amino acid substitution within the 302-325 region is a histidine/asparagine substitution, and the amino acid substitution within the 422-445 region is a phenylalanine/serine substitution.

As used herein, a naturally occurring *rhg*1 allele is any allele that encodes for a protein

15   having an extracellular LRR, a transmembrane domain, and STK domain where the naturally occurring allele is present on linkage group G and where certain *rhg*1 alleles, but not all *rhg*1 alleles, are capable of providing or contributing to resistance or partial resistance to a race of SCN. It is understood that such an allele can, using for example methods disclosed herein, be manipulated so that the nucleic acid molecule encoding the protein is no longer present on

20   linkage group G. It is also understood that such an allele can, using for example methods disclosed herein, be manipulated so that the nucleic acid molecule sequence is altered.

As used herein, an *rhg*1 SCN resistant allele is any *rhg*1 allele where that allele alone or in combination with other SCN resistant alleles present in the plant, such as an *Rhg*4 SCN resistant allele, provides resistance to a race of SCN, and that resistance is due, at least in part, to

25   the genetic contribution of the *rhg*1 allele.

SCN resistance or partial resistance is determined by a comparison of the plant in question with a known SCN sensitive host, Lee 74, according to the method set forth in Schmitt,

J. Nematol. 20:392-395 (1988). As used herein, resistance to a particular race of SCN is defined as having less than 10% of cyst development relative to the SCN sensitive host Lee 74. Moreover, as used herein, partial resistance to a particular race of SCN is defined as having more than 10% but less than 75% of cyst development relative to the SCN sensitive host Lee 74.

5   Any soybean plant having an *rhg*1 SCN resistant allele can be used in conjunction with the present invention. Soybeans with known *rhg*1 SCN resistant alleles can be used. Such soybeans include but are not limited to PI548402 (Peking), PI200499, A2869, Jack, A2069, PI209332 (No:4), PI404166 (Krasnoaarmejkaja), PI404198 (Sun huan do), PI437654 (Er-hej-jan), PI438489 (Chiquita), PI507354 (Tokei 421), PI548655 (Forrest), PI548988 (Pickett),

10   PI84751, PI437654, PI40792, Pyramid, Nathan, AG2201, A3469, AG3901, A3904, AG4301, AG4401, AG4501, AG4601, PION9492, PI88788, Dyer, Custer, Manokin, and Doles. In a preferred aspect, the soybean plant having an *rhg*1 SCN resistant allele is an *rhg*1 haplotype 2 allele. Examples of soybeans with an *rhg*1 haplotype 2 allele are PI548402 (Peking), PI404166 (Krasnoaarmejkaja), PI404198 (Sun huan do), PI437654 (Er-hej-jan), PI438489 (Chiquita),

15   PI507354 (Tokei 421), PI548655 (Forrest), PI548988 (Pickett), PI84751, PI437654, and PI40792. In addition, using the methods or agents of the present invention, soybeans and wild relative of soybean such as *Glycine soja* can be screened for the presence of *rhg*1 SCN resistant alleles.

        Any soybean plant having an *rhg*1 SCN sensitive allele can be used in conjunction with

20   the present invention. Such soybeans include A3244, A2833, AG3001, Williams, Will, A2704, Noir, DK23-51, Lee 74, Essex, Minsoy, A1923, and Hutcheson. In a preferred aspect, the soybean plant having an *rhg*1 SCN sensitive allele is an *rhg*1 A3244 allele. In addition, using the methods or agents of the present invention, soybeans and wild relatives of soybean such as *Glycine soja* can be screened for the presence of *rhg*1 SCN sensitive alleles.

25   Table 2, below, is a table showing single nucleotide polymorphisms (SNPs) and insertions/deletions (INDEL) sites for eight haplotype sequences of *rhg*1.

TABLE 2

Identification				Base number of contig 240O17_region_G3 of reference line A3244															
Hap	PI#	Line	Ph	45173	45309	45400	45416	45439	45611	45916	45958	46049	46113	46227	46703	47057	47140	47208
1	-	A3244	S	G	G	A	T	A	A	A	A	C	A	d1	0	T	C	C
2	PI548402	Peking	R	G	A	C	C	T	A	G	A	T	G	0	d2	C	C	C
3	PI423871	Toyosuzu	-	G	A	A	T	A	A	G	A	T	G	0	0	T	C	C
4	PI518672	Will	S	G	G	A	T	A	A	A	A	C	A	d1	0	T	A	T
5	-	A2704	S	G	G	A	T	A	A	A	A	C	A	d1	0	T	A	T
6	PI290136	Noir	S	A	A	A	C	T	G	A	T	T	A	0	d14	T	C	C
7	PI548658	Lee 74	S	A	A	A	C	T	G	A	T	T	A	0	d14	T	C	C
8	PI200499	-	R	G	A	A	C	A	A	A	A	T	A	0	d14	T	C	C
N/A	PI548667	Essex	S	A	A	A	C	T	G	A	T	T	A	0	d14	T	C	C
N/A	PI548389	Minsoy	S	G	G	A	T	A	A	A	A	C	A	d1	0	T	A	T
N/A	PI360843	Oshima.	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	T
N/A	-	A2869	R	-	-	-	-	-	-	-	-	-	-	-	0	d14	T	C	C
N/A	PI540556	Jack	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N/A	-	A2069	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N/A	PI209332	No.4	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2, continued

Identification				Base number of contig 240O17_region_G3 of reference line A3244															
Hap	PI#	Line	Ph	47571	47617	47796	47856	47937	48012	48060	48073	48135	48279	48413	48681	48881	49012	49316
1	-	A3244	S	G	C	A	T	T	T	C	C	A	C	G	A	0	A	T
2	PI548402	Peking	R	G	C	C	C	C	T	C	C	G	C	G	G	d19	G	T
3	PI423871	Toyosuzu	-	G	C	C	C	C	T	C	C	G	C	G	A	0	A	T
4	PI518672	Will	S	G	C	A	T	T	T	C	C	A	C	G	A	0	A	T
5	-	A2704	S	G	C	A	T	T	C	T	T	G	T	C	-	0	G	C
6	PI290136	Noir	S	A	A	C	C	C	C	T	T	G	T	C	G	0	G	C
7	PI548658	Lee 74	S	G	A	C	C	C	C	T	T	G	T	C	G	0	G	C
8	PI200499	-	R	G	A	C	C	C	C	T	T	G	T	C	G	0	G	C
N/A	PI548667	Essex	S	G	A	C	C	C	C	T	T	G	T	C	A/G	0	G	C

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N/A	PI548389	Minsoy	S	G	C	A	T	T	C/T	C/T	C/T	A	C	G	A	0	A	T
N/A	PI360843	Oshimas.	-	G	C	A	T/C	T/C	T	C	C	A/G	C	G	A	0	A	T
N/A	-	A2869	R	G	A	C	C	C	C	T	T	G	T	C	G	0	G	C
N/A	PI540556	Jack	R	-	-	C	C	C	C	T	T	G	T	C	G	0	G	C
N/A	-	A2069	R	-	-	C	T/C	T/C	C	T	T	A	T	C	G	0	G	C
N/A	PI209332	No.4	R	-	-	C	C	C	C	T	T	A/G	T	C	G	0	G	C

In Table 2, discrete haplotypes are designated 1 through 8. N/A refers to a haplotype that is not characterized. The Plant Introduction classification number is indicated in the "PI#" column. A dash indicates that no PI number is known or assigned for the line under investigation. The line from which the sequences are derived is indicated in the "line" column, with a dash indicating an unknown or unnamed line. The "Ph." (phenotype) column of table 2 indicates whether a given line has been reported as resistant (R) to at least one race of SCN or sensitive (S).

The nucleotide bases located at each of 30 positions in each of the haplotype sequences is shown in the columns labeled "Base number of contig 240017_region_G3 of reference line A3244." The base number at the top of each column corresponds to the base number in contig 240017_region_G3 of reference line A3224 (SEQ ID NOs: 2 and 3). The letters G, A, C, and T correspond to the bases guanine, adenine, cytosine, and thymine. Two bases separated by a slash (A/G, C/T, or T/C) indicate uncertainty at the specified position of the haplotype sequence. A "d" followed by a number indicates a deletion of a the length specified. That is, d1 is a one base deletion, d2 is a two base deletion, d14 is a fourteen base deletion, and d19 is a nineteen base deletion. A zero (0) indicates no deletion. A dash indicates that the identity of the base is undetermined.

Examination of table 2 reveals that the amino acid substitutions in the *rhg*1 coding region are common to the resistant lines PI467312 (Cha-mo-shi-dou), PI88788 and the southern susceptible lines Essex, Hutchenson, Noir and A1923. As used herein, a "southern" cultivar is any cultivar from maturity groups VI, VII, VIII, IX, or X, and a "northern" cultivar is any cultivar from maturity groups 000, 00, 0, I, II, III, IV, or V. This data is consistent with the

mapping experiments of Qui *et al.* (*Theor Appl Genet 98*:356-364 (1999)). Based on analysis of 200 F$_{2:3}$ families derived from a cross between Peking and Essex, the authors failed to detect any significant association with SCN resistance to races 1, 2, and 3, and the *rhg*1 locus on linkage group G. The authors point out that one of the markers, Bng122, which has been shown to have

5   significant linkage to *rhg*1 (Concibido *et al.*, *Crop Sci. 36*:1643-1650 (1996)), is not polymorphic in the population employed. It is also possible that the susceptible southern lines contain *rhg*1 and the susceptible phenotype reflects the polygenic nature of SCN resistance. In a study to uncover QTLs for sudden death syndrome (SDS) in soybean, two SCN resistant alleles originating from the susceptible parent Essex have been described (Hnetkovsky *et al.*, *Crop Sci.*

10   *36*:393-400).

Tables 3a, 3b, and 3c, below, show lines that share an *rhg*1 haplotype.

TABLE 3a

Haplotype 2 Lines		
PI#	Line	Ph.
PI548402	Peking	R
PI404166	Krasnoaarmejkaja	R
PI404198	(Sun huan do)	R
PI437654	Er-hej-jan	R
PI438489	(Chiquita)	R
PI507354	Tokei 421	R
PI548655	Forrest	R
PI548988	Pickett	R
PI84751	-	R
PI437654	-	R
PI40792	-	-

TABLE 3b

Haplotype 4 Lines		
PI#	Line	Ph.
-	Will	S
PI467312	Cha-mo-shi-dou	R
PI88788	-	R

TABLE 3c

Haplotype 6 Lines		
PI#	Line	Ph.
-	Noir	S
-	A1923	S
-	Hutcheson	S

In Tables 3a, 3b, and 3c, Plant Introduction classification number is indicated in the "PI#" column. A dash indicates that no PI number is known or assigned for the line in question. The line from which the sequences are derived is indicated in the "line" column, with a dash

5   indicating an unknown or unnamed line. The "Ph." column indicates whether a given line has been reported as resistant (R) to at least one race of SCN or sensitive (S), with a dash indicating that the phenotype is unknown.

In a preferred aspect, the source of either an *rhg*1 SCN sensitive allele or an *rhg*1 SCN resistant allele, or more preferably both, is an elite plant. An "elite line" is any line that has

10   resulted from breeding and selection for superior agronomic performance. Examples of elite lines are lines that are commercially available to farmers or soybean breeders such as HARTZ™ variety H4994, HARTZ™ variety H5218, HARTZ™ variety H5350, HARTZ™ variety H5545, HARTZ™ variety H5050, HARTZ™ variety H5454, HARTZ™ variety H5233, HARTZ™ variety H5488, HARTZ™ variety HLA572, HARTZ™ variety H6200, HARTZ™ variety H6104,

15   HARTZ™ variety H6255, HARTZ™ variety H6586, HARTZ™ variety H6191, HARTZ™ variety H7440, HARTZ™ variety H4452 Roundup Ready™, HARTZ™ variety H4994 Roundup Ready™, HARTZ™ variety H4988 Roundup Ready™, HARTZ™ variety H5000 Roundup Ready™, HARTZ™ variety H5147 Roundup Ready™, HARTZ™ variety H5247 Roundup Ready™, HARTZ™ variety H5350 Roundup Ready™, HARTZ™ variety H5545 Roundup

20   Ready™, HARTZ™ variety H5855 Roundup Ready™, HARTZ™ variety H5088 Roundup Ready™, HARTZ™ variety H5164 Roundup Ready™, HARTZ™ variety H5361 Roundup Ready™, HARTZ™ variety H5566 Roundup Ready™, HARTZ™ variety H5181 Roundup Ready™, HARTZ™ variety H5889 Roundup Ready™, HARTZ™ variety H5999 Roundup

Ready™, HARTZ™ variety H6013 Roundup Ready™, HARTZ™ variety H6255 Roundup Ready™, HARTZ™ variety H6454 Roundup Ready™, HARTZ™ variety H6686 Roundup Ready™, HARTZ™ variety H7152 Roundup Ready™, HARTZ™ variety H7550 Roundup Ready™, HARTZ™ variety H8001 Roundup Ready™ (HARTZ SEED, Stuttgart, Arkansas,

5 U.S.A.); A0868, AG0901, A1553, A1900, AG1901, A1923, A2069, AG2101, AG2201, A2247, AG2301, A2304, A2396, AG2401, AG2501, A2506, A2553, AG2701, AG2702, A2704, A2833, A2869, AG2901, AG2902, AG3001, AG3002, A3204, A3237, A3244, AG3301, AG3302, A3404, A3469, AG3502, A3559, AG3601, AG3701, AG3704, AG3750, A3834, AG3901, A3904, A4045 AG4301, A4341, AG4401, AG4501, AG4601, AG4602, A4604, AG4702,

10 AG4901, A4922, AG5401, A5547, AG5602, A5704, AG5801, AG5901, A5944, A5959, AG6101, QR4459 and QP4544 (Asgrow Seeds, Des Moines, Iowa, U.S.A.); DeKalb variety CX445 (DeKalb, Illinois). An elite plant is any plant from an elite line.

B)   *Rhg4*

The present invention provides a method for the production of a soybean plant having an

15 *Rhg4* SCN resistant allele comprising: (A) crossing a first soybean plant having an *Rhg4* SCN resistant allele with a second soybean plant having an *Rhg4* SCN sensitive allele to produce a segregating population; (B) screening the segregating population for a member having an *Rhg4* SCN resistant allele with a first nucleic acid molecule capable of specifically hybridizing to linkage group A2, wherein the first nucleic acid molecule specifically hybridizes to a second

20 nucleic acid molecule linked to the *Rhg4* SCN resistant allele; and, (C) selecting the member for further crossing and selection.

Rhg4 is located on linkage group A2 (Matson and Williams, *Crop Sci.* 5:447 (1965)). SCN resistant alleles of *Rhg4* provide partial resistance to SCN races 1 and 3 (U.S. Patent 5,491,081). Together, *rhg1* and *Rhg4* provide complete or nearly complete resistance to SCN

25 race 3. The dominant gene, *Rhg4*, was found to be closely linked to the seed coat color locus (*i*) (Matson and Williams, *Crop Sci.* 5:447 (1965)). The *i* locus in Peking was also reported to be linked with a recessive gene for resistance to SCN (Sugiyama and Katsumi, *Jpn. J. Breed.* 16:83-

86 (1966)). It is possible that Rhg4 and the recessive gene linked to the i locus are one and the same, which would call into question the classification of Rhg4 as a dominant gene.

Using bioinformatic approaches the Rhg4 coding region is predicted to contain 2 exons (coding coordinates 111805-113968 and 114684-115204 of SEQ ID NO: 4). Rhg4 encodes an

5   894 amino acid polypeptide. Rhg4 codes for a Xa21-like receptor kinase (SEQ ID NOs: 1099 and 1116-1119) (Song et al., Science 270, 1804-1806, (1995)). Rhg4 has an extracellular LRR domain (Rhg4, SEQ ID NO: 1099, residues 34-44), a transmembrane domain (Rhg4 SEQ ID NO: 1099, residues 449-471), and STK domain (Rhg4, SEQ ID NO: 1099, residues 531-830). In a preferred embodiment, the LRR domain has multiple LRR repeats. In a more preferred

10   embodiment, the LRR domain has 12 LRR repeats.

To identify proteins similar to the Rhg4 candidate, database searches are performed using the predicted peptide sequences. The Rhg4 candidate shows similarity to TMK (Y07748)(73.0% similarity and 54.8% identity (CLUSTALW (default parameters))) and TMK1 PRECURSOR

15   (70.6% similarity and 55.1% identity (CLUSTALW (default parameters))), which are rice and Arabidopsis receptor kinases, respectively. The predicted LRR extracellular domain reveals similarity to TMK (Y07748)(70.1% similarity and 46.6% identity (CLUSTALW (default parameters))), TMK1 PRECURSOR (g1707642) (65.8% similarity and 48.8% identity (CLUSTALW (default parameters))), and F21J9.1 (g2213607) (65.5% similarity and 45.6%

20   identity (CLUSTALW (default parameters))).

Figure 2 is an alignment of the LRR domain of the Rhg4 gene. A consensus sequence is shown as the top row. Each row of amino acids represents an LRR domain. The boxed region indicates the putative β-turn/ β-sheet structural motif postulated to be involved in ligand binding (Jones and Jones, Adv. Bot. Res. Incorp. Adv. Plant Path. 24;89-167 (1997)). The hydrophobic

25   leucine residues are thought to project into the core of the protein while the flanking amino acids are thought to be solvent exposed where they may interact with the ligand (Kobe and Deisenhofer, Nature 374; 183-186 (1995)). An "x" represents an arbitrary amino acid while an

"a" represents a hydrophobic residue (leucine, isoleucine, methionine, valine, or phenylalanine). Amino acid substitutions between resistant and sensitive phenotypes are bordered by a double line. The amino acid substitution within the 35-57 region is a histidine/glutamine substitution, and the amino acid substitution within the 81-104 region is a leucine/phenylalanine substitution.

5   As used herein, a naturally-occurring *Rhg4* allele is any allele that encodes for a protein having an extracellular LRR domain, a transmembrane domain, and STK domain where the naturally occurring allele is present on linkage group A2 and where certain *Rgh4* alleles, but not all *Rgh4* alleles, are capable of providing or contributing to resistance or partial resistance to a race of SCN. It is understood that such an allele can, using, for example methods disclosed

10   herein, be manipulated so that the nucleic acid molecule encoding the protein is no longer present on linkage group A2. It is also understood that such an allele can, using, for example methods disclosed herein, be manipulated so that the nucleic acid molecule sequence is altered.

        As used herein, an *Rhg4* SCN resistant allele is any *Rhg4* allele where that allele alone or in combination with other SCN resistant alleles present in the plant, such as an *rhg1* SCN

15   resistant allele, provides resistance to a race of SCN, and that resistance is due, at least in part, to the genetic contribution of the *Rhg4* allele.

        Any soybean plant having an *Rhg4* SCN resistant allele can be used in conjunction with the present invention. Soybeans with known *Rhg4* SCN resistant alleles can be used. Such soybeans include, but are not limited to, PI548402 (Peking), PI437654 (Er-hej-jan), PI438489

20   (Chiquita), PI507354 (Tokei 421), PI548655 (Forrest), PI548988 (Pickett), PI88788, PI404198 (Sun Huan Do), PI404166 (Krasnoaarmejkaja), Hartwig, Manokin, Doles, Dyer, and Custer. In a preferred aspect, the soybean plant having an *Rhg4* SCN resistant allele is an *Rhg4* haplotype 3 allele in a plant having either an *rhg1* haplotype 2 or *rhg1* haplotype 4 allele. Examples of soybeans with an *Rhg4* haplotype 3 allele are PI548402 (Peking), PI88788, PI404198 (Sun huan

25   do), PI438489 (Chiquita), PI437654 (Er-hej-jan), PI404166 (Krasnoaarmejkaja), PI548655 (Forrest), PI548988 (Pickett), and PI507354 (Tokei 421). In addition, using the methods or

agents of the present invention, soybeans and wild relatives of soybeans such as *Glycine soja* can

be screened for the presence of *Rhg*4 SCN resistant alleles.

Table 4 below is a table showing single nucleotide polymorphisms (SNPs) for three

haplotype sequences of *Rhg*4.

5

TABLE 4

Identification					Base number of contig 318O13_region_A3					Markers		
Hap	PI number	Line	Ph	Coat	111933	112065	112101	112461	114066	scn279	scnb267	scn273
1	-	A2069	R	yellow	T	A	T	A	T	2	2	2
1	-	A2869	R	yellow	T	A	T	A	T	2	2	2
1	-	A3244	S	yellow	T	A	T	A	T	2	2	2
1	PI87631	Kindaizu	R	yellow	T	A	T	A	T	2	2	2
1	PI548389	Minsoy	S	yellow	T	A	T	A	T	2	2	2
1	PI518664	Hutcheson	S	yellow	T	A	T	A	T	2	2	2
1	PI548658	Lee 74	S	yellow	T	A	T	A	T	-	2	2
2	PI540556	Jack	R	yellow	G	A	T	A	T	2	2	1
2	PI360843	Oshimashirome	R	yellow	G	A	T	A	T	-	-	-
2	PI423871	Toyosuzu	R	yellow	G	A	T	A	T	-	-	-
3	PI548402	Peking	R	black	G	C	C	T	G	1	1	1
3	PI88788	-	R	black	G	C	C	T	G	1	1	1
3	PI404198 B	(Sun huan do)	R	black	G	C	C	T	G	1	1	1
3	PI438489 B	(Chiquita)	R	black	G	C	C	T	G	1	1	1
3	PI437654	Er-hej-jan	R	black	G	C	C	T	G	2	1	1
3	PI404166	Krasnoaarmejkaja	R	black	G	C	C	T	G	1	1	-
3	PI290136	Noir	S	black	G	C	C	T	G	1	1	1
3	PI548655	Forrest	R	yellow	G	C	C	T	G	1	1	1
3	PI548988	Pickett	R	yellow	G	C	C	T	G	1	1	1
3	PI507354	Tokei 421	R	yellow	G	C	C	T	G	1	1	1
N/A	PI467312	Cha-mo-shi-dou	R	GnBr	G	C	C	T	-	1	1	1
N/A	PI209332	No.4	R	black	T	A	T	-	-	2	2	2
N/A	PI518672	Will	S	yellow	T	A	T	-	T	2	2	2
N/A	PI548667	Essex	S	yellow	T	A	T	-	T	2	2	2

In Table 4, discrete haplotypes are designated 1 through 3. N/A refers to a haplotype that

is not characterized. In Table 4, the Plant Introduction classification number is indicated in the

"PI#" column. A dash indicates that no PI number is known or assigned for the line under

investigation. The line from which the sequences are derived is indicated in the "line" column,

10   with a dash indicating an unknown or unnamed line. The "Ph." column of Table 4 indicates

whether a given line has been reported to be resistant (R) to at least one race of SCN, or sensitive

(S). The "coat" column shows the phenotypic coat color of a seed as either yellow, black, green/brown (GnBr), or unknown/unassigned (dash). At the *I* locus, black seeded varieties harbor the *i* allele for black or imperfect black seed coat. In a preferred embodiment, the seed has a yellow coat.

5   The nucleotide base located at each of 5 positions in each of the haplotype sequences is shown in the columns labeled "Base number of contig 318O13_region_A3." The base number at the top of each column correspond to the base number in the contig 318O13_region_A3 of reference line A3244 (SEQ ID NO: 4). The letters G, A, C, and T correspond to the bases guanine, adenine, cytosine, and thymine. A dash indicates that the identity of the base is

10   unknown.

The nucleotide base located at each of 5 positions in each of the haplotype sequences is shown in the columns labeled "Base number of contig 318O13_region_A3." The base number at the top of each column correspond to the base number in the contig 318O13_region_A3 of reference line A3244 (SEQ ID NO: 4). The letters G, A, C, and T correspond to the bases guanine, adenine, cytosine, and thymine. A dash indicates that the identity of the base is unknown.

Three different simple sequence repeat (SSR) or microsatellite markers that occur within the sequences, scn279 (SEQ ID NO: 292), scn267 (SEQ ID NO: 282), and scn273 (SEQ ID NO: 294), are listed under "markers." The allele of each marker occurring in a haplotype is indicated by a 1 or a 2, with a dash indicating that the information is not determined.

15   Any soybean plant having an *Rhg*4 SCN sensitive allele can be used in conjunction with the present invention. Such soybeans include A3244, Will, Noir, Lee 74, Essex, Minsoy, A2704, A2833, AG3001, Williams, DK23-51, and Hutcheson. In a preferred aspect, the soybean plant having an *Rhg*4 SCN sensitive allele is an *Rhg*4 A3244 allele. In addition, using the methods or agents of the present invention, soybeans and wild relative of soybean such as *Glycine soja* can

20   be screened for the presence of *Rhg*4 SCN sensitive alleles.

In a preferred aspect, the source of either an *Rhg*4 SCN sensitive allele or an *Rhg*4 SCN resistant allele, or more preferably both, is an elite plant.

In table 5, below, *rhg*1 and *Rhg*4 haplotypes for various cultivars are compared.

TABLE 5

Identification				Haplotype	
PI#	Line	Coat	Ph.	*rhg4*	*rhg1*
-	A3244	yellow	S	1	1
PI548402	Peking	black	R	3	2
PI404198 B	(Sun huan do)	black	R	3	2
PI438489 B	(Chiquita)	black	R	3	2
PI437654	Er-hej-jan	black	R	3	2
PI404166	Krasnoaarmejkaja	black	R	3	2
PI548655	Forrest	yellow	R	3	2
PI548988	Pickett	yellow	R	3	2
PI507354	Tokei 421	yellow	R	3	2
PI88788	-	black	R	3	4
PI467312	Cha-mo-shi-dou	GnBr	R	N/A	4
-	Noir	black	S	3	6
-	Jack	yellow	R	2	N/A
PI360843	Oshimashirome	yellow	R	2	N/A
PI423871	Toyosuzu	yellow	R	2	3
PI209332	No.4	black	R	N/A	N/A
PI87631	Kindaizu	yellow	R	1	-
-	Minsoy	yellow	S	1	N/A
-	Will	yellow	S	N/A	4
-	Hutcheson	yellow	S	1	6
-	Lee 74	yellow	S	N/A	7
-	Essex	yellow	S	N/A	N/A
-	A2069	yellow	R	1	N/A
-	A2869	yellow	R	1	N/A

In Table 5, haplotypes, as used in Tables 2 through 4, are listed for each line. N/A refers to a haplotype that is not characterized. The Plant Introduction classification number is indicated in the "PI#" column. A dash indicates that no PI number is known or assigned for the line under investigation. The line from which the sequences are derived is indicated in the "line" column, with a dash indicating an unknown or unnamed line. The "Ph." column of table 5 indicates whether a given line has been reported to be resistant (R) to at least one race of SCN, or sensitive

(S). The "coat" column shows the phenotypic coat color of a seed as either yellow, black, green/brown (GnBr), or unknown/unassigned (dash). At the *I* locus, black seeded varieties harbor the *i* allele for black or imperfect black seed coat. In a preferred embodiment, the seed has a yellow coat.

5   **Screening for *rhg*1 and *Rhg*4 alleles**

Any appropriate method can be used to screen for a plant having an *rhg*1 SCN resistant allele. Any appropriate method can be used to screen for a plant having an *Rhg*4 SCN resistant allele. In a preferred aspect of the present invention, a nucleic acid marker of the present invention can be used (see section entitled "Screening for *rhg*1 and *Rhg*4 alleles" and subsection

10   (ii) of the section entitled "Agents").

Additional markers, such as SSRs, AFLP markers, RFLP markers, RAPD markers, phenotypic markers, SNPs, isozyme markers, microarray transcription profiles that are genetically linked to or correlated with alleles of a QTL of the present invention can be utilized (Walton, *Seed World* 22-29 (July, 1993); Burow and Blake, *Molecular Dissection of Complex*

15   *Traits,* 13-29, Eds. Paterson, CRC Press, New York (1988)). Methods to isolate such markers are known in the art. For example, locus-specific SSRs can be obtained by screening a genomic library for SSRs, sequencing of "positive" clones, designing primers which flank the repeats, and amplifying genomic DNA with these primers. The size of the resulting amplification products can vary by integral numbers of the basic repeat unit. To detect a polymorphism, PCR products

20   can be radiolabeled, separated on denaturing polyacrylamide gels, and detected by autoradiography. Fragments with size differences >4 bp can also be resolved on agarose gels, thus avoiding radioactivity.

Other SSR markers may be utilized. Amplification of simple tandem repeats, mainly of the [CA]$_n$ type were reported by Litt and Luty, *Amer. J. Human Genet. 44*:397-401 (1989);

25   Smeets *et al., Human Genet. 83*:245-251 (1989); Tautz, *Nucleic Acids Res. 17*:6463-6472 (1989); Weber and May, *Am. J. Hum. Genet. 44*:388-396 (1989). Weber, *Genomics 7*:524-530 (1990), reported that the level of polymorphism detected by PCR-amplified [CA]$_n$ type SSRs

depends on the number of the "perfect" (*i.e.*, uninterrupted), tandemly repeated motifs. Below a certain threshold (*i.e.*, 12 CA-repeats), the SSRs were reported to be primarily monomorphic. Above this threshold, however, the probability of polymorphism increases with SSR length. Consequently, long, perfect arrays of SSRs are preferred for the generation of markers, *i.e.*, for

5   the design and synthesis of flanking primers.

       Suitable primers can be deduced from DNA databases (*e.g.*, Akkaya *et al.*, *Genetics.* *132*:1131-1139 (1992)). Alternatively, size-selected genomic libraries (200 to 500 bp) can be constructed by, for example, using the following steps: (1) isolation of genomic DNA; (2) digestion with one or more 4 base-specific restriction enzymes; (3) size-selection of restriction

10   fragments by agarose gel electrophoresis, excision and purification of the desire size fraction; (4) ligation of the DNA into a suitable vector and transformation into a suitable *E. coli* strain; (5) screening for the presence of SSRs by colony or plaque hybridization with a labeled probe; (6) isolation of positive clones and sequencing of the inserts; and (7) design of suitable primers flanking the SSR.

15   Establishing libraries with small, size-selected inserts can be advantageous for SSR isolation for two reasons: (1) long SSRs are often unstable in *E. coli*, and (2) positive clones can be sequenced without subcloning. A number of approaches have been reported for the enrichment of SSRs in genomic libraries. Such enrichment procedures are particularly useful if libraries are screened with comparatively rare tri- and tetranucleotide repeat motifs. One such

20   approach has been described by Ostrander *et al.*, *Proc. Natl. Acad. Sci. (U.S.A)*. *89*:3419-3423 (1992), who reported the generation of a small-insert phagemid library in an *E. coli* strain deficient in UTPase (d8t) and uracil-N-glycosylase (ung) genes. In the absence of UTPase and uracil-N-glycosylase, dUTP can compete with dTTP for the incorporation into DNA. Single-stranded phagemid DNA isolated from such a library can be primed with $[CA]_n$ and $[TG]_n$

25   primers for second strand synthesis, and the products used to transform a wild-type *E. coli* strain. Since under these conditions there will be selection against single-stranded, uracil-containing

DNA molecules, the resulting library will consist of primer-extended, double-stranded products and an about 50-fold enrichment in CA-repeats.

Other reported enrichment strategies rely on hybridization selection of simple sequence repeats prior to cloning (Karagyozov *et al.*, *Nucleic Acids Res.* *21*:3911-3912 (1993); Armour *et al.*, *Hum. Mol. Gen.* *3*:599-605 (1994); Kijas *et al.*, *Genome 38*:349-355 (1994); Kandpal *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 91*:88-92 (1994); Edwards *et al.*, *Am. J. Hum. Genet. 49*:746-756 (1991)). Hybridization selection, can for example, involve the following steps: (1) genomic DNA is fragmented, either by sonication, or by digestion with a restriction enzyme; (2) genomic DNA fragments are ligated to adapters that allow a "whole genome PCR" at this or a later stage of the procedure; (3) genomic DNA fragments are amplified, denatured and hybridized with single-stranded SSR sequences bound to a nylon membrane; (4) after washing off unbound DNA, hybridizing fragments enriched for SSRs are eluted from the membrane by boiling or alkali treatment, reamplified using adapter-complementary primers, and digested with a restriction enzyme to remove the adapters; and (5) DNA fragments are ligated into a suitable vector and transformed into a suitable *E. coli* strain. SSRs can be found in up to 50-70% of the clones obtained from these procedures (Armour *et al.*, *Hum. Mol. Gen.* *3*:599-605 (1994); Edwards *et al.*, *Am. J. Hum. Genet. 49*:746-756 (1991)).

An alternative hybridization selection strategy was reported by Kijas *et al.*, *Genome 38*:599-605 (1994), which replaced the nylon membrane with biotinylated, SSR-complementary oligonucleotides attached to streptavidin-coated magnetic particles. SSR-containing DNA fragments are selectively bound to the magnetic beads, reamplified, restriction-digested and cloned.

It is further understood that other additional markers on linkage group G or A2 may be utilized (Morgante *et al.*, *Genome 37*:763-769 (1994)). As used herein, reference to the linkage group of G or A2 refers to the linkage group that corresponds to linkage groups U5 and U3, respectively from the genetic map of *Glycine max* (Mansur *et al.*, *Crop Sci. 36:* 1327-1336 (1996), and linkage groups G and A2, respectively, of *Glycine max* x. *Glycine soja* (Shoemaker

et al., Genetics 144: 329-336 (1996)) that is present in *Glycine soja* (Soybase, an Agricultural

Research Service, United States Department of Agriculture (http://129.186.26.940/ and USDA -

Agricultural Research Service: http://www.ars.usda.gov/)).

PCR-amplified SSRs can be used, because they are locus-specific, codominant, occur in

5   large numbers and allow the unambiguous identification of alleles. Standard PCR-amplified

SSR protocols use radioisotopes and denaturing polyacrylamide gels to detect amplified SSRs.

In many situations, however, allele sizes are sufficiently different to be resolved on high

percentage agarose gels in combination with ethidium bromide staining (Bell and Ecker,

*Genomics 19:*137-144 (1994); Becker and Heun, *Genome 38:*991-998 (1995); Huttel, Ph.D.

10   Thesis, University of Frankfurt, Germany (1996)). High resolution without applying

radioactivity is also provided by nondenaturing polyacrylamide gels in combination with either

ethidium bromide (Scrimshaw, *Biotechniques 13:*2189 (1992)) or silver straining (Klinkicht and

Tautz, *Molecular Ecology 1:* 133-134 (1992); Neilan *et al., Biotechniques 17:*708-712 (1994)).

An alternative of PCR-amplified SSRs typing involves the use of fluorescent primers in

15   combination with a semi-automated DNA sequencer (Schwengel *et al., Genomics 22:*46-54

(1994)). Fluorescent PCR products can be detected by real-time laser scanning during gel

electrophoresis. An advantage of this technology is that different amplification reactions as well

as a size marker (each labeled with a different fluorophore) can be combined into one lane during

electrophoresis. Multiplex analysis of up to 24 different SSR loci per lane has been reported

20   (Schwengel *et al., Genomics 22:*46-54 (1994)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the

use of nucleic acid amplification methods. Such methods specifically increase the concentration

of polynucleotides that span the polymorphic site, or include that site and sequences located

either distal or proximal to it. Such amplified molecules can be readily detected by gel

25   electrophoresis or other means.

The most preferred method of achieving such amplification employs the polymerase

chain reaction ("PCR") (Mullis *et al., Cold Spring Harbor Symp. Quant. Biol. 51:*263-273

(1986); Erlich *et al.*, European Patent Appln. 50,424; European Patent Appln. 84,796, European Patent Application 258,017, European Patent Appln. 237,362; Mullis, European Patent Appln. 201,184; Mullis *et al.*, U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki *et al.*, U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal

5   sequences that define a polymorphism in its double-stranded form.

In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" ("LCR") may be used (Barany, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:189-193 (1991)). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides is selected to permit the pair to hybridize to abutting sequences of the same

10   strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a polymorphic site. In one embodiment, either oligonucleotide will be

15   designed to include the actual polymorphic site of the polymorphism. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains or lacks the specific nucleotide that is complementary to the polymorphic site present on the oligonucleotide. Alternatively, the oligonucleotides may be selected such that they do not include the polymorphic site (see, Segev, PCT Application WO

20   90/01069).

The "Oligonucleotide Ligation Assay" ("OLA") may alternatively be employed (Landegren *et al.*, *Science 241*:1077-1080 (1988)). The OLA protocol uses two oligonucleotides that are designed to be capable of hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR,

25   however, OLA results in "linear" rather than exponential amplification of the target sequence.

Nickerson *et al.* have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8923-8927 (1990)). In this

method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA. In addition to requiring multiple, and separate, processing steps, one problem associated with such combinations is that they inherit all of the problems associated with PCR and OLA.

5   Schemes based on ligation of two (or more) oligonucleotides in the presence of a nucleic acid having the sequence of the resulting "di-oligonucleotide," thereby amplifying the di-oligonucleotide, are also known (Wu *et al.*, *Genomics 4*:560-569 (1989)), and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers,

10   branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (Malek *et al.*, U.S. Patent 5,130,238; Davey *et al.*, European Patent Application 329,822; Schuster *et al.*, U.S. Patent 5,169,766; Miller *et al.*, PCT Patent Application WO 89/06700; Kwoh, *et al.*, *Proc. Natl. Acad. Sci.* (U.S.A.) *86*:1173-1177 (1989); Gingeras *et al.*, PCT Patent Application WO

15   88/10315; Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 89*:392-396 (1992)).

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis. SSCP is a method capable of identifying most sequence variations in a single strand of DNA, typically between 150 and 250 nucleotides in length (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996); Orita *et*

20   *al.*, *Genomics 5:* 874-879 (1989)). Under denaturing conditions a single strand of DNA will adopt a conformation that is uniquely dependent on its sequence conformation. This conformation usually will be different, even if only a single base is changed. Most conformations have been reported to alter the physical configuration or size sufficiently to be detectable by electrophoresis. A number of protocols have been described for SSCP including,

25   but not limited to, Lee *et al.*, *Anal. Biochem. 205:* 289-293 (1992); Suzuki *et al.*, *Anal. Biochem. 192:* 82-84 (1991); Lo *et al.*, *Nucleic Acids Research 20:* 1005-1009 (1992); Sarkar *et al.*, *Genomics 13:*441-443 (1992). It is understood that one or more of the nucleic acids of the

present invention can be utilized as markers or probes to detect polymorphisms by SSCP analysis.

Polymorphisms may also be found using random amplified polymorphic DNA (RAPD) (Williams *et al., Nucl. Acids Res. 18*: 6531-6535 (1990)) and cleaveable amplified polymorphic sequences (CAPS) (Lyamichev *et al., Science 260*: 778-783 (1993)). It is understood that one or more of the nucleic acid molecules of the present invention can be utilized as markers or probes to detect polymorphisms by RAPD or CAPS analysis.

The identification of a polymorphism can be determined in a variety of ways. By correlating the presence or absence of it in a plant with the presence or absence of a phenotype, it is possible to predict the phenotype of that plant. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (*e.g.*, a variable nucleotide tandem repeat (VNTR) polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, individuals that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and plant genetic analyses (Glassberg, UK Patent Application 2135774; Skolnick *et al., Cytogen. Cell Genet. 32*:58-67 (1982); Botstein *et al., Ann. J. Hum. Genet. 32*:314-331 (1980); Fischer *et al.* (PCT Application WO90/13668); Uhlen, PCT Application WO90/11369)).

A central attribute of "single nucleotide polymorphisms," or "SNPs" is that the site of the polymorphism is at a single nucleotide. SNPs have certain reported advantages over RFLPs and VNTRs. First, SNPs are more stable than other classes of polymorphisms. Their spontaneous mutation rate is approximately 10^{-9} (Kornberg, DNA Replication, W.H. Freeman & Co., San Francisco, 1980), approximately 1,000 times less frequent than VNTRs (U.S. Patent 5,679,524). Second, SNPs occur at greater frequency, and with greater uniformity than RFLPs and VNTRs. As SNPs result from sequence variation, new polymorphisms can be identified by sequencing random genomic or cDNA molecules. SNPs can also result from deletions, point mutations and

insertions. Any single base alteration, whatever the cause, can be an SNP. The greater

frequency of SNPs means that they can be more readily identified than the other classes of

polymorphisms.

SNPs and insertion/deletions can be detected by methods, by any of a variety of methods

5 including those disclosed in U.S. Patents 5,210,015; 5,876,930 and 6,030,787 in which an

oligonucleotide probe having reporter and quencher molecules is hybridized to a target

polynucleotide. The probe is degraded by 5' → 3' exonuclease activity of a nucleic acid

polymerase. A useful assay is available from AB Biosystems (850 Lincoln Centre Drive, Foster

City, CA) as the Taqman® assay.

10 Specific nucleotide variations such as SNPs and insertion/deletions can also be detected

by labeled base extension methods as disclosed in U.S. Patents 6,004,744; 6,013,431; 5,595,890;

5,762,876; and 5,945,283. These methods are based on primer extension and incorporation of

detectable nucleoside triphosphates. The primer is designed to anneal to the sequence

immediately adjacent to the variable nucleotide which can be can be detected after incorporation

15 of as few as one labeled nucleoside triphosphate. US Patent 5,468,613 discloses allele specific

oligonucleotide hybridizations where single or multiple nucleotide variations in nucleic acid

sequence can be detected in nucleic acids by a process in which the sequence containing the

nucleotide variation is amplified, spotted on a membrane and treated with a labeled sequence-

specific oligonucleotide probe.

20 Such methods also include the direct or indirect sequencing of the site, the use of

restriction enzymes where the respective alleles of the site create or destroy a restriction site, the

use of allele-specific hybridization probes, the use of antibodies that are specific for the proteins

encoded by the different alleles of the polymorphism or by other biochemical interpretation.

SNPs can be sequenced by a number of methods. Two basic methods may be used for DNA

25 sequencing, the chain termination method of Sanger *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 74:*

5463-5467 (1977), and the chemical degradation method of Maxam and Gilbert, *Proc. Nat.*

Acad. Sci. (U.S.A.) 74: 560-564 (1977). Automation and advances in technology such as the

replacement of radioisotopes with fluorescence-based sequencing have reduced the effort required to sequence DNA (Craxton, *Methods, 2:* 20-26 (1991); Ju *et al., Proc. Natl. Acad. Sci. (U.S.A.) 92:* 4347-4351 (1995); Tabor and Richardson, *Proc. Natl. Acad. Sci. (U.S.A.) 92:* 6339-6343 (1995)). Automated sequencers are available from, for example, Pharmacia Biotech, Inc.,

5   Piscataway, New Jersey (Pharmacia ALF), LI-COR, Inc., Lincoln, Nebraska (LI-COR 4,000) and Millipore, Bedford, Massachusetts (Millipore BaseStation).

In addition, advances in capillary gel electrophoresis have also reduced the effort required to sequence DNA and such advances provide a rapid high resolution approach for sequencing DNA samples (Swerdlow and Gesteland, *Nucleic Acids Res. 18*:1415-1419 (1990);

10   Smith, *Nature 349*:812-813 (1991); Luckey *et al., Methods Enzymol. 218*:154-172 (1993); Lu *et al., J. Chromatog. A. 680*:497-501 (1994); Carson *et al., Anal. Chem. 65*:3219-3226 (1993); Huang *et al., Anal. Chem. 64*:2149-2154 (1992); Kheterpal *et al., Electrophoresis 17*:1852-1859 (1996); Quesada and Zhang, *Electrophoresis 17*:1841-1851 (1996); Baba, *Yakugaku Zasshi 117*:265-281 (1997), Marino, *Appl. Theor. Electrophor.* 5:1-5 (1995)).

15   The genetic linkage of marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics, 121*:185-199 (1989), and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics, 121*:185-199 (1989), and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling*

20   *Quantitative Traits Using MAPMAKER/QTL,* Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Version 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated,

25   together with an MLE assuming no QTL effect, to avoid false positives. A $\log_{10}$ of an odds ratio (LOD) is then calculated as: LOD = $\log_{10}$ (MLE for the presence of a QTL/MLE given no linked QTL).

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein,

5   *Genetics, 121*:185-199 (1989), and further described by Arús and Moreno-González, *Plant Breeding*, Hayward, Bosemark, Romagosa (eds.) Chapman & Hall, London, pp. 314-331 (1993).

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use of non-parametric methods (Kruglyak and Lander, *Genetics, 139*:1421-1428 (1995)). Multiple regression methods or models can be

10   also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breed*, van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*, Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative

15   QTL at a given marker interval, and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics, 136*:1447-1455 (1994) and Zeng, *Genetics, 136*:1457-1468 (1994). Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding,* van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in

20   Plant Breeding, The Netherlands, pp.195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng, *Genetics, 136*:1457-1468 (1994)). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al., Theo. Appl. Genet. 91*:33-37 (1995)).

Selection of an appropriate mapping or segregation populations is important to map

25   construction. The choice of appropriate mapping population depends on the type of marker systems employed (Tanksley *et al., Molecular mapping plant chromosomes. Chromosome structure and function: Impact of new concepts* J.P. Gustafson and R. Appels (eds.), Plenum

Press, New York, pp. 157-173 (1988)). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating

5   populations with a relatively large array of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

As used herein, the progeny include not only, without limitation, the products of any cross (be it a backcross or otherwise) between two plants, but all progeny whose pedigree traces back to the original cross. Specifically, without limitation, such progeny include plants that have

10   12.5% or less genetic material derived from one of the two originally crossed plants. As used herein, a second plant is derived from a first plant if the second plant's pedigree includes the first plant.

An F_2 population is the first generation of selfing after the hybrid seed is produced. Usually a single F_1 plant is selfed to generate a population segregating for all the genes in

15   Mendelian (1:2:1) fashion. Maximum genetic information is obtained from a completely classified F_2 population using a codominant marker system (Mather, Measurement of Linkage in Heredity: Methuen and Co., (1938)). In the case of dominant markers, progeny tests (e.g., F_3, BCF_2) are required to identify the heterozygotes, thus making it equivalent to a completely classified F_2 population. However, this procedure is often prohibitive because of the cost and

20   time involved in progeny testing. Progeny testing of F_2 individuals is often used in map construction where phenotypes do not consistently reflect genotype (e.g., disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations (e.g., F_3 or BCF_2) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations (F_2, F_3), where linkage groups

25   have not been completely disassociated by recombination events (i.e., maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually >F_5, developed from continuously selfing F_2 lines towards homozygosity) can be used as a mapping population.

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Information obtained from dominant markers can be maximized by using RIL because all loci

are homozygous or nearly so. Under conditions of tight linkage (*i.e.*, about <10%

recombination), dominant and co-dominant markers evaluated in RIL populations provide more

information per individual than either marker type in backcross populations (Reiter *et al., Proc.*

5   *Natl. Acad. Sci. (U.S.A.) 89*:1477-1481 (1992)). However, as the distance between markers

becomes larger (*i.e.*, loci become more independent), the information in RIL populations

decreases dramatically when compared to codominant markers.

Backcross populations (*e.g.*, generated from a cross between a successful variety

(recurrent parent) and another variety (donor parent) carrying a trait not present in the former)

10   can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be

made to recover most of its desirable traits. Thus a population is created consisting of

individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic

of genomic regions from the donor parent. Backcross populations can be useful for mapping

dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent

15   parent have contrasting polymorphic marker alleles (Reiter *et al., Proc. Natl. Acad. Sci. (U.S.A.)*

89:1477-1481 (1992)). Information obtained from backcross populations using either

codominant or dominant markers is less than that obtained from F_2 populations because one,

rather than two, recombinant gametes are sampled per plant. Backcross populations, however,

are more informative (at low marker saturation) when compared to RILs as the distance between

20   linked loci increases in RIL populations (*i.e.*, about .15% recombination). Increased

recombination can be beneficial for resolution of tight linkages, but may be undesirable in the

construction of maps with low marker saturation.

Near-isogenic lines (NIL) created by many backcrosses to produce an array of individuals

that are nearly identical in genetic composition except for the trait or genomic region under

25   interrogation can be used as a mapping population. In mapping with NILs, only a portion of the

polymorphic loci are expected to map to a selected region.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of

linkage between markers and traits of interest (Michelmore, *et al., Proc. Natl. Acad. Sci. (U.S.A.)*

88:9828-9832 (1991)). In BSA, two bulked DNA samples are drawn from a segregating

population originating from a single cross. These bulks contain individuals that are identical for

5   a particular trait (resistant or sensitive to particular disease) or genomic region but arbitrary at

unlinked regions (*i.e.,* heterozygous). Regions unlinked to the target region will not differ

between the bulked samples of many individuals in BSA.

Plants generated using a method of the present invention can be part of or generated from

a breeding program. The choice of breeding method depends on the mode of plant reproduction,

10   the heritability of the trait(s) being improved, and the type of cultivar used commercially (*e.g.,* F_1

hybrid cultivar, pureline cultivar, etc). Selected, non-limiting approaches, for breeding the plants

of the present invention are set forth below. A breeding program can be enhanced using marker

assisted selection of the progeny of any cross. It is further understood that any commercial and

non-commercial cultivars can be utilized in a breeding program. Factors such as, for example,

15   emergence vigor, vegetative vigor, stress tolerance, disease resistance, branching, flowering,

seed set, seed size, seed density, standability, and threshability *etc.* will generally dictate the

choice.

For highly heritable traits, a choice of superior individual plants evaluated at a single

location will be effective, whereas for traits with low heritability, selection should be based on

20   mean values obtained from replicated evaluations of families of related plants. Popular selection

methods commonly include pedigree selection, modified pedigree selection, mass selection, and

recurrent selection. In a preferred embodiment a backcross or recurrent breeding program is

undertaken.

The complexity of inheritance influences choice of the breeding method. Backcross

25   breeding can be used to transfer one or a few favorable genes for a highly heritable trait into a

desirable cultivar. This approach has been used extensively for breeding disease-resistant

cultivars. Various recurrent selection techniques are used to improve quantitatively inherited

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traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross.

Breeding lines can be tested and compared to appropriate standards in environments representative of the commercial target area(s) for two or more generations. The best lines are candidates for new commercial cultivars; those still deficient in traits may be used as parents to produce new populations for further selection.

One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations can provide a better estimate of its genetic worth. A breeder can select and cross two or more parental lines, followed by repeated selfing and selection, producing many new genetic combinations.

The development of new soybean cultivars requires the development and selection of soybean varieties, the crossing of these varieties and selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids are selected for certain single gene traits such as pod color, flower color, seed yield, pubescence color or herbicide resistance which indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods can be used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. New cultivars can be evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents who possess favorable, complementary traits are crossed to produce an F_1. An F_2 population is produced by selfing one or several F_1's. Selection of the best individuals in the best

families is performed. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (*i.e.*, F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

5   Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of

10   the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting parent is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the

15   next generation. When the population has been advanced from the F_2 to the desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance

20   is completed.

In a multiple-seed procedure, soybean breeders commonly harvest one or more pods from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique.

25   The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each by hand for the single-

seed procedure. The multiple-seed procedure also makes it possible to plant the same number of

seeds of a population each generation of inbreeding.

Descriptions of other breeding methods that are commonly used for different traits and

crops can be found in one of several reference books (e.g., Fehr, *Principles of Cultivar*

5   *Development* Vol. 1, pp. 2-3 (1987)).

In a preferred aspect of the present invention the source of the *rhg*1 SCN resistant allele

for use in a breeding program is derived from a plant selected from the group consisting of

PI548402 (Peking), PI200499, A2869, Jack, A2069, PI209332 (No:4), PI404166

(Krasnoaarmejkaja), PI404198 (Sun huan do), PI437654 (Er-hej-jan), PI438489 (Chiquita),

10   PI507354 (Tokei 421), PI548655 (Forrest), PI548988 (Pickett), PI84751, PI437654, PI40792,

Pyramid, Nathan, AG2201, A3469, AG3901, A3904, AG4301, AG4401, AG4501, AG4601,

PION9492, PI88788, Dyer, Custer, Manokin, Doles, and SCN resistant progeny thereof (USDA,

Soybean Germplasm Collection, University of Illinois, Illinois). In a more preferred aspect, the

source of the *rhg*1 SCN resistant allele for use in a breeding program is derived from a plant

15   selected from the group consisting of PI548402 (Peking), PI404166 (Krasnoaarmejkaja),

PI404198 (Sun huan do), PI437654 (Er-hej-jan), PI438489 (Chiquita), PI507354 (Tokei 421),

PI548655 (Forrest), PI548988 (Pickett), PI84751, PI437654, PI40792, and SCN resistant

progeny thereof.

In a preferred aspect of the present invention the source of the *rhg*1 SCN sensitive allele

20   for use in a breeding program is derived from a plant selected from the group consisting of

A3244, A2833, AG3001, Williams, Will, A2704, Noir, DK23-51, Lee 74, Essex, Minsoy,

A1923, Hutcheson, and SCN sensitive progeny thereof. In a more preferred aspect, the source of

the *rhg*1 SCN sensitive allele for use in a breeding program is derived from an A3244 plant, and

SCN sensitive progeny thereof.

25   In a preferred aspect of the present invention the source of the *Rhg*4 SCN resistant allele

for use in a breeding program is derived from a plant selected from the group consisting of

PI548402 (Peking), PI437654 (Er-hej-jan), PI438489 (Chiquita), PI507354 (Tokei 421),

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PI548655 (Forrest), PI548988 (Pickett), PI88788, PI404198 (Sun Huan Do), PI404166

(Krasnoaarmejkaja), Hartwig, Manokin, Doles, Dyer, Custer, and SCN resistant progeny thereof.

In a more preferred aspect, the source of the *Rhg*4 SCN resistant allele for use in a breeding

program is derived from a plant selected from the group consisting of PI548402 (Peking),

5   PI88788, PI404198 (Sun huan do), PI438489 (Chiquita), PI437654 (Er-hej-jan), PI404166

(Krasnoaarmejkaja), PI548655 (Forrest), PI548988 (Pickett), PI507354 (Tokei 421), and SCN

resistant progeny thereof.

       In a preferred aspect of the present invention the source of the *Rhg*4 SCN sensitive allele

for use in a breeding program is derived from a plant selected from the group consisting of

10   A3244, Will, Noir, Lee 74, Essex, Minsoy, A2704, A2833, AG3001, Williams, DK23-51, and

Hutcheson, and SCN sensitive progeny thereof. In a more preferred aspect, the source of the

*Rhg*4 SCN sensitive allele for use in a breeding program is derived from an A3244 plant, and

SCN sensitive progeny thereof.

       As used herein linkage of a nucleic acid sequence with another nucleic acid sequence

15   may be genetic or physical. In a preferred embodiment, a nucleic acid marker is genetically

linked to either *rhg*1 or *Rhg*4, where the marker nucleic acid molecule exhibits a LOD score of

greater than 2.0, as judged by interval mapping, for SCN resistance or partial resistance,

preferably where the marker nucleic acid molecule exhibits a LOD score of greater than 3.0, as

judged by interval mapping, for SCN resistance or partial resistance, more preferably where the

20   marker nucleic acid molecule exhibits a LOD score of greater than 3.5, as judged by interval

mapping, for SCN resistance or partial resistance and even more preferably where the marker

nucleic acid molecule exhibits a LOD score of about 4.0, as judged by interval mapping, for

SCN resistance or partial resistance based on maximum likelihood methods described by Lander

and Botstein, *Genetics, 121*:185-199 (1989), and implemented in the software package

25   MAPMAKER/QTL (default parameters)(Lincoln and Lander, *Mapping Genes Controlling*

Quantitative Traits Using MAPMAKER/QTL, Whitehead Institute for Biomedical Research,

Massachusetts, (1990)).

In another embodiment the nucleic acid molecule may be physically linked to either *rhg*1 or *Rhg*4. In a preferred embodiment, the nucleic acid marker specifically hybridizes to a nucleic acid molecule having a sequence that is present on linkage group G within 500 kb or 100kb, more preferably within 50kb, even more preferably within 25kb of an *rhg*1 allele, where the *rgh*1

5   allele is preferably a sensitive allele, and more preferably a sensitive allele from A3244. In a preferred embodiment the nucleic acid marker is capable of specifically hybridizing to a nucleic acid molecule having a sequence that is present on linkage group A2 within 500kb or 100kb, more preferably within 50kb, even more preferably within 25kb of an *Rhg*4 allele, where the *Rgh*4 allele is preferably a sensitive allele, and more preferably a sensitive allele from A3244.

10   The present invention provides a method of investigating an *rhg*1 haplotype of a soybean plant comprising: (A) isolating nucleic acid molecules from the soybean plant; (B) determining the nucleic acid sequence of an *rhg*1 allele or part thereof; and, (C) comparing the nucleic acid sequence of the *rhg*1 allele or part thereof to a reference nucleic acid sequence.

As used herein, the term "investigating" refers to any method capable of detecting a

15   feature, such as a polymorphism or haplotype. Nucleic acid molecules only need to be isolated from a soybean plant to the degree of purity necessary for the task required or to a greater purity if desired. A person of ordinary skill in the art has available techniques to isolate nucleic acid molecules from plants to a sufficient purity, for example without limitation, to sequence the desired region of the nucleic acid molecule or to carry out a marker assay.

20   The determination of an *rhg*1or *Rhg*4 allele or part thereof may be carried out using any technique. Illustration of such techniques include techniques that provide the nucleic acid sequence for an *rhg*1 or *rhg*4 allele or part thereof include amplification of a desired allele or part thereof (see, for example, the Examples and SEQ ID NOs: 8-53). In a preferred embodiment, the nucleic acid sequence determined is that of an exon of an *rhg*1 allele, more

25   preferably exon 1 or exon 3 of an *rhg*1 allele, or of an LRR domain. In another preferred embodiment, a single nucleotide is determined. In another preferred embodiment, the nucleic acid sequence determined is that of an LRR domain.

A comparison of a sequence with a reference sequence can be carried out with any appropriate sequence comparison method.

As used herein, a reference sequence is any *rhg*1 allele sequence or consensus sequence. A reference sequence may be a nucleic acid sequence or an amino acid sequence. In a preferred embodiment, the reference sequence is any SCN resistant *rhg*1 allele sequence. In a further preferred embodiment, the *rhg*1 reference sequence is selected from the group consisting of SEQ ID NOs: 2, 3, 5, 6, 8-23, 28-43, 1097, 1098, and 1100-1115.

The present invention provides a method of investigating an *Rhg*4 haplotype of a soybean plant comprising: (A) isolating nucleic acid molecules from the soybean plant; (B) determining the nucleic acid sequence of an *Rhg*4 allele or part thereof; and (C) comparing the nucleic acid sequence of the *Rhg*4 allele or part thereof to a reference nucleic acid sequence.

As used herein, a reference sequence is any *Rhg*4 allele sequence or consensus sequence. A reference sequence ma be a nucleic acid sequence or an amino acid sequence. In a preferred embodiment, the reference sequence is any SCN resistant *Rhg*4 allele sequence. In a further preferred embodiment, the *Rhg*4 reference sequence is selected from the group consisting of SEQ ID NOs: 4, 7, 44-47, 50-53, 1099, and 1116-1119.

The present invention provides a method of introgressing SCN resistance or partial SCN resistance into a soybean plant comprising: performing marker assisted selection of the soybean plant with a nucleic acid marker, wherein the nucleic acid marker specifically hybridizes with a nucleic acid molecule having a first nucleic acid sequence that is physically linked to a second nucleic acid sequence that is located on linkage group G of soybean A3244, wherein the second nucleic acid sequence is within 500 kb of a third nucleic acid sequence which is capable of specifically hybridizing with the nucleic acid sequence of SEQ ID NO: 5, 6, complements thereof, or fragments thereof; and, selecting the soybean plant based on the marker assisted selection.

The present invention provides a method of introgressing SCN resistance or partial SCN resistance into a soybean plant comprising: performing marker assisted selection of the soybean

plant with a nucleic acid marker, wherein the nucleic acid marker specifically hybridizes with a nucleic acid molecule having a first nucleic acid sequence that is physically linked to a second nucleic acid sequence that is located on linkage group A2 of soybean A3244, wherein the second nucleic acid sequence is within 500 kb of a third nucleic acid sequence which is capable of

5   specifically hybridizing with the nucleic acid sequence of SEQ ID NO: 7, complements thereor, or fragments thereof; and, selecting the soybean plant based on the marker assisted selection. Marker assisted introgression of traits into plants has been reported. Marker assisted introgression involves the transfer of a chromosome region defined by one or more markers from one germplasm to a second germplasm. In a preferred embodiment the introgression is carried

10   out by backcrossing with an *rhg*1 or *Rhg*4 SCN resistant soybean recurrent parent.

In light of the current disclosure, plant introductions and germplasm can be screened with a marker nucleic acid molecule of the present invention to screen for alleles of *rhg*1 or *Rhg*4 using one or more of techniques disclosed herein or known in the art.

The present invention also provides for parts of the plants produced by a method of the

15   present invention. Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred embodiment of the present invention, the plant part is a seed.

. Plants or parts thereof produced by a method of the present invention may be grown in culture and regenerated. Methods for the regeneration of soybean plants from various tissue types and methods for the tissue culture of soybean are known in the art (See, for example,

20   Widholm *et al., In Vitro Selection and Culture-induced Variation in Soybean,* In Soybean: Genetics, Molecular Biology and Biotechnology, Eds. Verma and Shoemaker, CAB International, Wallingford, Oxon, England (1996)). Regeneration techniques for plants such as soybean can use as the starting material a variety of tissue or cell types. With soybean in particular, regeneration processes have been developed that begin with certain differentiated

25   tissue types such as meristems, Cartha *et al., Can. J. Bot. 59:*1671-1679 (1981), hypocotyl sections, Cameya *et al., Plant Science Letters 21:* 289-294 (1981), and stem node segments, Saka *et al., Plant Science Letters, 19:* 193-201 (1980); Cheng *et al., Plant Science Letters, 19:*

91-99 (1980). Regeneration of whole sexually mature soybean plants from somatic embryos generated from explants of immature soybean embryos has been reported (Ranch *et al., In Vitro Cellular & Developmental Biology 21*: 653-658 (1985). Regeneration of mature soybean plants from tissue culture by organogenesis and embryogenesis has also been reported (Barwale *et al., Planta 167*: 473-481 (1986); Wright *et al., Plant Cell Reports 5*: 150-154 (1986)).

Agents

One skilled in the art can refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include *Current Protocols in Molecular Biology* Ausubel, *et al.*, eds., John Wiley & Sons, N.Y. (1989), and supplements through September (1998), *Molecular Cloning, A Laboratory Manual*, Sambrook *et al*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), *Genome Analysis: A Laboratory Manual 1: Analyzing DNA*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1997); *Genome Analysis: A Laboratory Manual 2: Detecting Genes*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1998); *Genome Analysis: A Laboratory Manual 3: Cloning Systems*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Genome Analysis: A Laboratory Manual 4: Mapping Genomes*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); Plant Molecular Biology: A Laboratory Manual, Clark, Springer-Verlag, Berlin, (1997), *Methods in Plant Molecular Biology*, Maliga *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1995). These texts can, of course, also be referred to in making or using an aspect of the invention. It is understood that any of the agents of the invention can be substantially purified and/or be biologically active and/or recombinant.

(a)   **Nucleic Acid Molecules**

Nucleic acid molecules of the present invention include, without limitation, nucleic acid molecules having a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-1096 and complements thereof. A subset of the nucleic acid molecules of the present invention includes nucleic acid molecules that encode a protein or fragment thereof. Another subset of the

nucleic acid molecules of the present invention are cDNA molecules. Another subset of the nucleic acid molecules of the present invention includes nucleic acid molecules that are marker molecules. A further subset of the nucleic acid molecules of the present invention are those nucleic acid molecules having promoter sequences.

5   Fragment nucleic acid molecules may comprise significant portion(s) of, or indeed most of, these nucleic acid molecules. In preferred embodiments, the fragments may comprise smaller polynucleotides, *e.g.*, oligonucleotides having from about 20 to about 250 nucleotide residues and more preferably, about 20 to about 100 nucleotide residues, or about 40 to about 60 nucleotide residues. In another preferred embodiment, fragment molecules may be at least 15

10   nucleotides, at least 30 nucleotides, at least 50 nucleotides, or at least 100 nucleotides.

The term "substantially purified," as used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native state. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more

15   preferably 90% free, and most preferably 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native state.

The agents of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another

20   nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response.

The agents of the present invention may also be recombinant. As used herein, the term recombinant describes (a) nucleic acid molecules that are constructed or modified outside of

25   cells and that can replicate or function in a living cell, (b) molecules that result from the transcription, replication or translation of recombinant nucleic acid molecules, or (c) organisms

that contain recombinant nucleic acid molecules or are modified using recombinant nucleic acid molecules.

It is understood that the agents of the present invention may be labeled with reagents that facilitate detection of the agent, *e.g.*, fluorescent labels, (Prober *et al.*, *Science 238*:336-340

5   (1987); Albarella *et al.*, EP 144914), chemical labels, (Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417), and modified bases, (Miyoshi *et al.*, EP 119448) including nucleotides with radioactive elements, *e.g.*, ^{32}P, ^{33}P, ^{35}S or ^{125}I, such as ^{32}P dCTP.

It is further understood, that the present invention provides recombinant bacterial, animal, fungal and plant cells and viral constructs comprising the agents of the present invention.

10   Nucleic acid molecules or fragments thereof of the present invention are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is said to be the "complement" of another nucleic acid

15   molecule if they exhibit "complete complementarity," *i.e.*, each nucleotide in one sequence is complementary to its base pairing partner nucleotide in another sequence. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can

20   hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Nucleic acid molecules which hybridize to other nucleic acid molecules, *e.g.*, at least under low stringency conditions are said to be "hybridizable cognates" of the other nucleic acid molecules. Conventional stringency conditions are described by Sambrook *et al.*, *Molecular Cloning*, A Laboratory Manual, 2nd Ed.,

25   Cold Spring Harbor Press, Cold Spring Harbor, New York (1989) and by Haymes *et al.*, *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not

completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

5   Appropriate stringency conditions which promote DNA hybridization, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 X SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 50°C to a high stringency of

10   about 0.2 X SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

      In a preferred embodiment, a nucleic acid of the present invention will specifically

15   hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 1096 or complements thereof under moderately stringent conditions, for example at about 2.0 X SSC and about 65°C.

      In a particularly preferred embodiment, a nucleic acid of the present invention will include those nucleic acid molecules that specifically hybridize to one or more of the nucleic

20   acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 1096 or complements thereof under high stringency conditions such as 0.2 X SSC and about 65°C.

      In one aspect of the present invention, the nucleic acid molecules of the present invention comprise one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 1096 or complements thereof or fragments of either. In another aspect of the present

25   invention, one or more of the nucleic acid molecules of the present invention share at least 60% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 1096 or complements thereof or fragments of either. In a further aspect of

the present invention, one or more of the nucleic acid molecules of the present invention share at least 70% or more, *e.g.,* at least 80%, sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 1096 or complements thereof or fragments of either. In a more preferred aspect of the present invention, one or more of the

5   nucleic acid molecules of the present invention share at least 90% or more, *e.g.,* at least 95% and up to 100% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 1096 complements thereof or fragments of either.

As used herein "sequence identity" refers to the extent to which two optimally aligned polynucleotide or peptide sequences are invariant throughout a window of alignment of

10   components, *e.g.,* nucleotides or amino acids. An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in reference sequence segment, *i.e.,* the entire reference sequence or a smaller defined part of the reference sequence. "Percent identity" is the identity fraction times 100.

15   Useful methods for determining sequence identity are disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073. More particularly, preferred computer programs for determining sequence identity include the Basic Local Alignment Search Tool (BLAST) programs which are publicly available from National Center Biotechnology Information (NCBI)

20   at the National Library of Medicine, National Institute of Health, Bethesda, Md. 20894; see BLAST Manual, Altschul *et al.,* NCBI, NLM, NIH; Altschul *et al., J. Mol. Biol. 215:*403-410 (1990); version 2.0 or higher of BLAST programs allows the introduction of gaps (deletions and insertions) into alignments; BLASTX can be used to determine sequence identity between a polynucleotide sequence query and a protein sequence database; and, BLASTN can be used to

25   determine sequence identity between between sequences.

For purposes of this invention "percent identity" shall be determined using BLASTX version 2.0.14 (default parameters), BLASTN version 2.0.14, or BLASTP 2.0.14.

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A particularly preferred group of nucleic acid sequences are those present in the soybean insert of the clones set forth in table 6 below.

TABLE 6

Line	Names of Clones Containing the Specified Gene		
	*Rhg*4	*rhg*1/frag 1	*rhg*1/frag 2
Forrest	Forrest 1	Forrest 7	Forrest13
Peking	Peking 1	Peking 7	Peking 13
Pickett	Pickett 1	Pickett 7	Pickett 13
PI84751	PI 84751.1	PI 84751.7	PI 84751.13
PI87631	PI 87631.1	PI 87631.7	PI 87631.13
PI87631-1	PI 87631-1.1		PI 87631-1.13
PI88788R	PI 88788R.1	PI 88788R.7	PI 88788R.13
PI89772			PI 89772.13
PI90763		PI 90763.7	PI 90763.13
PI200499	PI 200499.1	PI 200499.7	PI 200499.13
PI209332	PI 209332.1		PI 209332.13
PI404166	PI 404166.1	PI 404166.7	PI 404166.13
PI404198A		PI 404198A.7	PI 404198A.13
PI404198B	PI 404198B.1	PI 404198B.7	PI404198B.13
PI437654	PI 437654.1	PI 437654.7	PI 437654.13
PI438489B	PI 438489.1	PI 438489.7	PI 438489B.13
PI467312	PI 467312.1	PI 467312.7	PI 467312.13
PI507354	PI 507354.1	PI 507354.7	PI 507354.13
PI423871	PI 423871.1	PI 423871.7	PI 423871.13
PI407922		PI 407922.7	PI 407922.13
PI360843	PI 360843.1	PI 360843.7	PI 360843.13
A2869	A2869.1	A 2869.7	A2869.13
A2069	A2069.1		A2069.13
Jack	JACK1		JACK13
Will	WILL1	WILL.7	WILL13
Minsoy	Minsoy1	Minsoy.7	MINSOY13
Noir	Noir1	Noir.7	NOIR13
Hutcheson	Hutcheson1	Hutcheson.7	Hutcheson.13
A1923	A1923.1	A1923.7	A1923.13

Line	Names of Clones Containing the Specified Gene		
	Rhg4	*rhg1*/frag 1	*rhg1*/frag.2
A2704		A2704.7	A2704.13
Essex	Essex1	Essex.7	ESSEX13
A3244	A3244.1	A 3244.7	A3244.13
Lee74	Lee74.1	Lee74.7	Lee74.13
PI437654		R107C17.7	R107C17.13

Table 5 shows clones comprising *rhg*1 and *Rhg*4 sequences. The "Lines" column indicates the cultivar from which the sequence in the clone is derived. The *Rhg*4, *rhg*1/frag1, and *rhg*1/frag2 columns show the clones derived from the lines that have the *Rhg*4, *rhg*1 fragment 1, or *rhg*1 fragment 2, respectively. *Rhg*4 is amplified with SEQ ID NOs: 48 and 49, which produces a 3.5 kb product. *rhg*1 fragment 1 is amplified with SEQ ID NOs: 24 and 25, which produces a 2.9 kb product, and *rhg*1 fragment 2 is amplified with SEQ ID NOs: 26 and 27, which produces a 1.75 kb product. All fragments are subcloned into a pCR4-TOPO vector.

(i)   Nucleic Acid Molecules Encoding Proteins or Fragments Thereof

A)   *rhg*1

The present invention includes nucleic acid molecules that code for an *rhg*1 protein or fragment thereof. Examples of such nucleic acid molecules include those that code for the proteins set forth in SEQ ID NOs: 1097, 1100, 1098, 1101, and 1102-1115. Examples of illustrative fragment molecules include, without limitation, an extracellular LRR domain (*rhg*1, *v.1*, SEQ ID NO: 1097, residues 164-457; *rhg*1, *v.2*, SEQ ID NO: 1098, residues 141-434), a transmembrane domain (*rhg*1, *v.1*, SEQ ID NO: 1097, residues 508-530; *rhg*1, *v.2*, SEQ ID NO: 1098, residues 33-51 and 485-507), and an STK domain (*rhg*1, *v.1*, SEQ ID NO: 1097, residues 578-869; *rhg*1, *v.2*, SEQ ID NO: 1098, residues 555-846). Examples of illustrative nucleic acid molecules include SEQ ID NOs: 5, 6, 8-23, and 28-43.

B) *Rhg*4

The present invention includes nucleic acid molecules that code for an *Rhg*4 protein or fragment thereof. Examples of such nucleic acid molecules include those that code for the

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proteins set forth in SEQ ID NOs: 1099 and 1116-1119. Examples of illustrative fragment molecules include, without limitation, an extracellular LRR domain (SEQ ID NO: 1099, residues 34-44), a transmembrane domain (SEQ ID NO: 1099, residues 449-471), and an STK domain (SEQ ID NO: 1099, residues 531-830). Examples of illustrative nucleic acid molecules include

5   SEQ ID NOs: 7, 44-47, and 50-53.

C)   *Rhg*1 and *Rhg*4

In another further aspect of the present invention, nucleic acid molecules of the present invention can comprise sequences which differ from those encoding a protein or fragment thereof in SEQ ID NO: 1097 through SEQ ID NO: 1119 due to fact that the different nucleic acid

10   sequence encodes a protein having one or more conservative amino acid changes. It is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

It is well known in the art that one or more amino acids in a native sequence can be substituted with another amino acid(s), the charge and polarity of which are similar to that of the

15   native amino acid, *i.e.*, a conservative amino acid substitution. Conserved substitutions for an amino acid within the native polypeptide sequence can be selected from other members of the class to which the naturally occurring amino acid belongs. Amino acids can be divided into the following four groups: (1) acidic amino acids, (2) basic amino acids, (3) neutral polar amino acids, and (4) neutral nonpolar amino acids. Representative amino acids within these various

20   groups include, but are not limited to: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, cystine, tyrosine, asparagine, and glutamine; and (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

25   Conservative amino acid changes within the native polypeptides sequence can be made by substituting one amino acid within one of these groups with another amino acid within the same group. Biologically functional equivalents of the proteins or fragments thereof of the

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present invention can have ten or fewer conservative amino acid changes, more preferably seven

or fewer conservative amino acid changes, and most preferably five or fewer conservative amino

acid changes. The encoding nucleotide sequence will thus have corresponding base

substitutions, permitting it to encode biologically functional equivalent forms of the proteins or

5   fragments of the present invention.

It is understood that certain amino acids may be substituted for other amino acids in a

protein structure without appreciable loss of interactive binding capacity with structures such as,

for example, antigen-binding regions of antibodies or binding sites on substrate molecules.

Because it is the interactive capacity and nature of a protein that defines that protein's biological

10   functional activity, certain amino acid sequence substitutions can be made in a protein sequence

and, of course, its underlying DNA coding sequence and, nevertheless, obtain a protein with like

properties. It is thus contemplated by the inventors that various changes may be made in the

peptide sequences of the proteins or fragments of the present invention, or corresponding DNA

sequences that encode said peptides, without appreciable loss of their biological utility or

15   activity. It is understood that codons capable of coding for such amino acid changes are known

in the art.

In making such changes, the hydropathic index of amino acids may be considered. The

importance of the hydropathic amino acid index in conferring interactive biological function on a

protein is generally understood in the art (Kyte and Doolittle, *J. Mol. Biol. 157*, 105-132 (1982).

20   It is accepted that the relative hydropathic character of the amino acid contributes to the

secondary structure of the resultant protein, which in turn defines the interaction of the protein

with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens,

and the like. In making such changes, the substitution of amino acids whose hydropathic indices

are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within

25   ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made

effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, states that the greatest local

average hydrophilicity of a protein, as govern by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those encoding a peptide set forth in SEQ ID NO: 1097 through SEQ ID NO: 1119 or

5   fragment thereof due to the fact that one or more codons encoding an amino acid has been substituted for a codon that encodes a nonessential substitution of the amino acid originally encoded.

Agents of the invention include nucleic acid molecules that encode at least about a contiguous 10 amino acid region of a protein of the present invention, more preferably at least

10   about a contiguous 11 to 14 or larger amino acid region of a protein of the present invention. It is understood that the present invention includes nucleic acid molecules that specifically hybridize or exhibit a particular identity to the nucleic acid molecules described in (i). *See* (a) above.

(ii)   Nucleic Acid Molecule Markers and Collections of Such Molecules

One aspect of the present invention concerns nucleic acid molecules of the present invention that can act as markers. As used herein, a "marker" is an indicator for the presence of at least one phenotype or polymorphism, such as single nucleotide polymorphisms (SNPs), cleaveable amplified polymorphic sequences (CAPS), amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs),

20   or random amplified polymorphic DNA (RAPDs). A "nucleic acid marker" as used herein means a nucleic acid molecule that is capable of being a marker for detecting a polymorphism or phenotype.

In one embodiment of the present invention, the nucleic acid marker specifically hybridizes to a nucleic acid molecule having a nucleic acid sequence selected from the group

25   SEQ NOs: 1-1096 and complements thereof. In a preferred embodiment, the nucleic acid marker is capable of detecting an *rhg*1 SNP or INDEL set forth in table 2. In a preferred embodiment,

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the nucleic acid marker is capable of detecting an *Rgh*4 SNP or INDEL set forth in table 4. In another preferred embodiment the nucleic acid marker is a nucleic acid molecule capable of acting as a PCR primer to amplify an *rhg*1 or *Rhg*4 coding region. Examples of such primers include, without limitation, nucleic acid molecules having a nucleic acid sequence set forth in

5   SEQ ID NO: 401 –1096 and complements thereof. Such primers can be used in pairs to amplify a region (amplicons, e.g., without limitation, SEQ ID NOs: 54-400) that can be further investigated using techniques known in the art such as nucleic acid sequencing. Preferred pairs are those with identical "Seq ID" (*see* Description of the Sequence Listing) except for the fact that one "Seq ID" recites forward primer and one recites reverse primer.

10   In another embodiment of the present invention, the nucleic acid marker specifically hybridizes to a nucleic acid molecule having a sequence that is present on linkage group G within 500 kb or 100kb, more preferably within 50kb, even more preferably within 25kb of an *rhg*1 allele, where the *Rgh4* allele is preferably a sensitive allele, and more preferably a sensitive allele from A3244. In a preferred embodiment the nucleic acid marker specifically hybridizes to

15   a nucleic acid molecule having a sequence that is present on linkage group A2 within 500kb or 100kb, more preferably within 50kb, even more preferably within 25kb of an *Rhg*4 allele, where the *Rgh*4 allele is preferably a sensitive allele, and more preferably a sensitive allele from A3244.

As used herein, a "collection of nucleic acid molecules" is a population of nucleic acid

20   molecules where at least two, preferably all, of the nucleic acid molecules differ, at least in part, in their nucleic acid sequence. It is understood, that as used herein, an individual species within a collection of nucleic acid molecules may be physically separate or alternatively not physically separate from one or more other species within the collection of nucleic acid molecules. An example of a situation where individual species may be physically separate but considered a

25   collection of nucleic acid molecules is where more than two species are present in a single location such as an array.

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As used herein, where a collection of nucleic acid molecules is a marker for a particular attribute, the level, pattern, occurrence and/or absence of the nucleic acid molecules associated with the attribute are not required to be the same between species of the collection. For example, the increase in the level of a species when in combination with the decrease in a second species

5   could be diagnostic for a particular attribute. In a preferred embodiment of the present invention, the level, pattern, occurrence and/or absence of a nucleic acid molecule and/or collection of nucleic acid molecules of the present invention is a marker for SCN resistance.

In one embodiment, the marker is any nucleic acid molecule that specifically hybridizes to any nucleic acid sequence set forth herein. In another embodiment, the marker is a marker

10   capable of distinguishing among the haplotypes of either *rhg*1 or *Rhg*4. In yet another embodiment, more than one marker is used to simultaneously distinguish more than one haplotype. In a preferred embodiment, two, three, four, six, eight, twenty five or fifty or more nucleic acid markers are used simultaneously. In another embodiment, one or more markers that are capable of distinguishing among the haplotypes of *rhg*1 and one or more markers that are

15   capable of distinguishing among the haplotypes of *Rhg*4 are used together.

(iii)   **Nucleic acid molecules having promoter sequences and other regulatory sequences**

The present invention includes nucleic acid molecules that are an *rhg*1 or *Rhg*4 promoter

20   or fragment thereof. Examples of such nucleic acid molecules include those set forth in SEQ ID NO: 2, upstream of coordinate 45163 and SEQ ID NO: 3, upstream of coordinate 46798. As used herein a promoter is a nucleic acid sequence that when joined with a coding region is capable of expressing the protein or fragment thereof so encoded. In a preferred embodiment the promoter sequence corresponds to between 500 nucleotides and 5,000 nucleotides or between

25   300 nucleotides and 700 nucleotides of the nucleic acid sequence set forth in SEQ ID NO: 2 between coordinates 45163 and 40163, or SEQ ID NO:3 between coordinates 46798 and 41798 , or the nucleic acid sequence set forth in SEQ ID NO: 4 between coordinates 111805 and 106805

Preferred partial promoter regions include the TATA box region, e.g. at coordinates 44234 through 44246 of SEQ ID NO: 2 and at coordinates107826 through 107835 of SEQ ID NO: 4, and CAAT box region, e.g. at coordinates 106243 through 106259 of SEQ ID NO: 4.

Other regulatory sequences include introns or 3' untranslated regions (3'UTRs)

5   associated with *rhg*1 and *Rhg*4. In a preferred embodiment, an intron is selected from a nucleic acid comprising a sequence selected from SEQ ID NO: 2 (*rhg*1 *v.1* at coordinates 45315-45449, 45510-46940, and 48764-48974), SEQ ID NO: 3 (*rhg*1 *v.2* at coordinates 48764-48974) and SEQ ID NO: 4 (*Rhg*4 at coordinates 113969-114683). In another preferred embodiment, a 3'UTR is located within 5,000 nucleotides, more preferable within 1000 nucleotides in the 3'

10   direction of the last coding nucleotide of either *rhg*1 or *Rhg*4 (SEQ ID NO: 2, *rhg*1 *v.1*, coordinate 49573, SEQ ID NO: 3, *rhg*1, *v.2*, coordinate 49573, SEQ ID NO: 4, *Rhg*4, coordinate 115204).

It is understood that the present invention includes nucleic acid molecules that specifically hybridize or exhibit a particular identity to the nucleic acid molecules described in

15   (iii). *See* (a) above.

(b)   Protein and Peptide Molecules

A class of agents comprises one or more of the protein or peptide molecules encoded by SEQ ID NO: 1097 through SEQ ID NO:1119 or one or more of the protein or fragment thereof or peptide molecules encoded by other nucleic acid agents of the present invention. As used

20   herein, the term "protein molecule" and "peptide molecule" mean any protein or protein fragment or peptide or polypeptide molecule that comprises ten or more amino acids, preferably at least 11 or 12 or more, more preferably at least 13 or 14 amino acids. It is well know in the art that proteins may undergo modification, including post-translational modifications, such as, but not limited to, disulfide bond formation, glycosylation, phosphorylation, or oligomerization.

25   Thus, as used herein, the terms "protein molecule" and "peptide molecule" include molecules that are modified by any biological or non-biological process. The terms "amino acid" and

"amino acids" refer to all naturally occurring L-amino acids. This definition is meant to include norleucine, ornithine, homocysteine, and homoserine.

One or more of the protein or peptide molecules may be produced via chemical synthesis, or more preferably, by expression in a suitable bacterial or eukaryotic host. Suitable methods for
5   expression are described by Sambrook, *et al.*, (In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989), or similar texts.

Another class of agents comprise protein or peptide molecules encoded by SEQ ID NO: 1097 through SEQ ID NO: 1119 or complements thereof or, fragments or fusions thereof in which non-essential, or not relevant, amino acid residues have been added, replaced, or deleted.
10   An example of such a homolog is a protein homolog of each soybean species, including but not limited to alfalfa, barley, *Brassica*, broccoli, cabbage, citrus, garlic, oat, oilseed rape, onion, canola, flax, pea, peanut, pepper, potato, rye, soybean, strawberry, sugarcane, sugarbeet, soybean, maize, rice, cotton, sorghum, *Arabidopsis*, wheat, pine, fir, eucalyptus, apple, lettuce, peas, lentils, grape, banana, tea, turf grasses, *etc.* Particularly preferred non- soybean plants to
15   utilize for the isolation of homologs would include alfalfa, barley, oat, oilseed rape, canola, ornamentals, sugarcane, sugarbeet, soybean, maize, rice, cotton, sorghum, *Arabidopsis*, wheat, potato, and turf grasses. Such a homolog can be obtained by any of a variety of methods. Most preferably, as indicated above, one or more of the disclosed sequences (SEQ ID NO: 1 through SEQ ID NO: 1096 or complements thereof) will be used to define a pair of primers that may be
20   used to isolate the protein homolog-encoding nucleic acid molecules from any desired species. Such molecules can be expressed to yield protein homologs by recombinant means.

      (c)   **Plant Constructs and Plant Transformants**

One or more of the nucleic acid molecules of the invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell
25   and the plant cell regenerated into a whole, fertile or sterile plant. Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism. In a preferred embodiment the exogenous genetic material

includes a nucleic acid molecule of the present invention, preferably a nucleic acid molecule

having at least 20 nucleotides of a sequence selected from the group consisting of SEQ ID NO: 1

through SEQ ID NO: 1096 and complements thereof. In a preferred embodiment, the nucleic

acid molecule codes for a protein or fragment thereof described in Section (i). In another

5   preferred embodiment, the nucleic acid molecule is a promoter or fragment thereof described in

Section (iii).

Such genetic material may be transferred into either monocotyledons and dicotyledons

including, but not limited to tomato, eggplant, maize, soybean, *Arabidopsis,* phaseolus, peanut,

alfalfa, wheat, rice, oat, sorghum, rye, tritordeum, millet, fescue, perennial ryegrass, sugarcane,

10   cranberry, papaya, banana, banana, muskmelon, apple, cucumber, dendrobium, gladiolus,

chrysanthemum, liliacea, cotton, eucalyptus, sunflower, canola, turfgrass, sugarbeet, coffee and

dioscorea (Christou, In: *Particle Bombardment for Genetic Engineering of Plants,*

Biotechnology Intelligence Unit. Academic Press, San Diego, California (1996).

In a preferred embodiment, the genetic material is transferred to a soybean. Preferred

15   soybeans to transfer an *rhg*1 SCN resistance allele are selected from the group consisting of

PI548402 (Peking), PI200499, A2869, Jack, A2069, PI209332 (No:4), PI404166

(Krasnoaarmejkaja), PI404198 (Sun huan do), PI437654 (Er-hej-jan), PI438489 (Chiquita),

PI507354 (Tokei 421), PI548655 (Forrest), PI548988 (Pickett), PI84751, PI437654, PI40792,

Pyramid, Nathan, AG2201, A3469, AG3901, A3904, AG4301, AG4401, AG4501, AG4601,

20   PION9492, PI88788, Dyer, Custer, Manokin, and Doles.

Preferred soybeans to transfer an *Rhg*4 SCN resistance allele are selected from the group

consisting of PI548402 (Peking), PI437654 (Er-hej-jan), PI438489 (Chiquita), PI507354 (Tokei

421), PI548655 (Forrest), PI548988 (Pickett), PI88788, PI404198 (Sun Huan Do), PI404166

(Krasnoaarmejkaja), Hartwig, Manokin, Doles, Dyer, and Custer.

25   Transfer of a nucleic acid that encodes for a protein can result in overexpression of that

protein in a transformed cell or transgenic plant. One or more of the proteins or fragments

thereof encoded by nucleic acid molecules of the invention may be overexpressed in a

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transformed cell or transformed plant. Such overexpression may be the result of transient or stable transfer of the exogenous genetic material. Such overexpression can also result in SCN resistance to one or more races of SCN.

Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (*See, Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springier, New York (1997).

A construct or vector may include a plant promoter to express the protein or protein fragment of choice. A number of promoters, which are active in plant cells, have been described in the literature. These include the nopaline synthase (NOS) promoter (Ebert *et al., Proc. Natl. Acad. Sci. (U.S.A.) 84*:5745-5749 (1987), the octopine synthase (OCS) promoter (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al., Plant Mol. Biol. 9*:315-324 (1987), and the CaMV 35S promoter (Odell *et al., Nature 313*:810-812 (1985), the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al., Proc. Natl. Acad. Sci. (U.S.A.) 84*:6624-6628 (1987), the sucrose synthase promoter (Yang *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87*:4144-4148 (1990), the R gene complex promoter (Chandler *et al., The Plant Cell 1*:1175-1183 (1989), and the chlorophyll a/b binding protein gene promoter, *etc.* These promoters have been used to create DNA constructs that have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913. The CaMV 35S promoters are preferred for use in plants. Promoters known or found to cause transcription of DNA in plant cells can be used in the invention.

For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is preferred that the promoters utilized have relatively high expression in these specific tissues. Tissue-specific expression of a protein of the present invention is a particularly preferred embodiment. For this purpose, one may choose from a number of promoters for genes with

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tissue- or cell-specific or -enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea (Edwards *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:3459-3463 (1990), the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat (Lloyd *et al.*, *Mol. Gen. Genet.* 225:209-216 (1991), the nuclear

5   photosynthetic ST-LS1 promoter from potato (Stockhaus *et al.*, *EMBO J.* 8:2445-2451 (1989), the STK (PAL) promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (*Larix laricina*), the promoter for the *cab* gene, cab6, from pine (Yamamoto *et al.*, *Plant Cell Physiol.* 35:773-778 (1994), the promoter for the

10   Cab-1 gene from wheat (Fejes *et al.*, *Plant Mol. Biol.* 15:921-932 (1990), the promoter for the CAB-1 gene from spinach (Lubberstedt *et al.*, *Plant Physiol.* 104:997-1006 (1994), the promoter for the cab1R gene from rice (Luan *et al.*, *Plant Cell.* 4:971-981 (1992), the pyruvate, orthophosphate dikinase (PPDK) promoter from maize (Matsuoka *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 90: 9586-9590 (1993), the promoter for the tobacco Lhcb1*2 gene (Cerdan *et al.*, *Plant*

15   *Mol. Biol.* 33:245-255 (1997), the *Arabidopsis thaliana* SUC2 sucrose-H+ symporter promoter (Truernit *et al.*, *Planta.* 196:564-570 (1995), and the promoter for the thylakoid membrane proteins from spinach (psaD, psaF, psaE, PC, FNR, atpC, atpD, cab, rbcS). Other promoters for the chlorophyll a/b-binding proteins may also be utilized in the invention, such as the promoters for LhcB gene and PsbP gene from white mustard (*Sinapis alba*; Kretsch *et al.*, *Plant Mol. Biol.*

20   28:219-229 (1995)).

For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of maize, wheat, rice and barley, it is preferred that the promoters utilized in the invention have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or -enhanced expression are known, including

25   the class I patatin promoter (Bevan *et al.*, *EMBO J.* 8:1899-1906 (1986); Jefferson *et al.*, *Plant Mol. Biol.* 14:995-1006 (1990)), the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter (Salanoubat and Belliard, *Gene* 60:47-56

(1987), Salanoubat and Belliard, *Gene 84*:181-185 (1989)), the promoter for the major tuber proteins including the 22 kd protein complexes and proteinase inhibitors (Hannapel, *Plant Physiol. 101*:703-704 (1993)), the promoter for the granule bound starch synthase gene (GBSS) (Visser *et al., Plant Mol. Biol. 17*:691-699 (1991)), and other class I and II patatins promoters

5   (Koster-Topfer *et al., Mol Gen Genet. 219*:390-396 (1989); Mignery *et al., Gene. 62*:27-44 (1988)).

Other promoters can also be used to express a protein or fragment thereof in specific tissues, such as seeds or fruits. The promoter for β-conglycinin (Chen *et al., Dev. Genet. 10*: 112-122 (1989)) or other seed-specific promoters such as the napin and phaseolin promoters, can

10   be used. The zeins are a group of storage proteins found in maize endosperm. Genomic clones for zein genes have been isolated (Pedersen *et al., Cell 29*:1015-1026 (1982)) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and genes, could also be used. Other promoters known to function, for example, in maize include the promoters for the following genes: *waxy, Brittle, Shrunken 2*, Branching enzymes I and II, starch synthases,

15   debranching enzymes, oleosins, glutelins and sucrose synthases. A particularly preferred promoter for maize endosperm expression is the promoter for the glutelin gene from rice, more particularly the Osgt-1 promoter (Zheng *et al., Mol. Cell Biol. 13*:5829-5842 (1993)). Examples of promoters suitable for expression in wheat include those promoters for the ADPglucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching

20   and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. A particularly preferred promoter is the promoter for rice glutelin, Osgt-1. Examples of such promoters for barley include those for the ADPGPP subunits,

25   the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins.

Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene (Samac *et al.*, *Plant Mol. Biol.* 25:587-596 (1994)). Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV35S promoter that have been identified (Lam *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)*

5   86:7890-7894 (1989)). Other root cell specific promoters include those reported by Conkling *et al.* (Conkling *et al.*, *Plant Physiol.* 93:1203-1211 (1990)).

Additional promoters that may be utilized are described, for example, in U.S. Patent Nos. 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436. In addition, a tissue specific enhancer may be used (Fromm *et al.*,

10 *The Plant Cell* 1:977-984 (1989)).

Preferred promoters are those set forth in Section (a)(iii) of Agents.

Constructs or vectors may also include, with the coding region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. A number of such sequences have been isolated, including the Tr7 3' sequence and the NOS 3' sequence

15 (Ingelbrecht *et al.*, *The Plant Cell* 1:671-680 (1989); Bevan *et al.*, *Nucleic Acids Res.* 11:369-385 (1983)).

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al.*, *Genes and Develop.* 1:1183-1200 (1987)), the sucrose synthase intron (Vasil *et al.*, *Plant Physiol.* 91:1575-1579 (1989)) and the TMV omega element (Gallie *et*

20 *al.*, *The Plant Cell* 1:301-311 (1989)). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to: a neomycin phosphotransferase gene (U.S. Patent

25 5,034,322), which codes for kanamycin resistance and can be selected for using kanamycin, G418, *etc.*; a bar gene which codes for bialaphos resistance; genes which encode glyphosate resistance (U.S. Patents 4,940,835; 5,188,642; 4,971,908; 5,627,061); a nitrilase gene which

confers resistance to bromoxynil (Stalker *et al., J. Biol. Chem. 263*:6310-6314 (1988)); a mutant

acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance

(European Patent Application 154,204 (Sept. 11, 1985)); and a methotrexate resistant DHFR

gene (Thillet *et al., J. Biol. Chem. 263*:12500-12508 (1988)).

5   A vector or construct may also include DNA sequence which encodes a transit peptide.

Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent

Application Publication Number 0218571). Translational enhancers may also be incorporated as

part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader

sequences which may serve to enhance expression of the gene products from the resulting

10   mRNA transcripts. Such sequences may be derived from the promoter selected to express the

gene or can be specifically modified to increase translation of the mRNA. Such regions may

also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene

sequence. For a review of optimizing expression of transgenes, see Koziel *et al., Plant Mol.

Biol. 32*:393-405 (1996).

15   A vector or construct may also include a screenable marker. Screenable markers may be

used to monitor expression. Exemplary screenable markers include: a β-glucuronidase or uidA

gene (GUS) which encodes an enzyme for which various chromogenic substrates are known

(Jefferson, *Plant Mol. Biol, Rep. 5*:387-405 (1987); Jefferson *et al., EMBO J. 6*:3901-3907

(1987)); an R-locus gene, which encodes a product that regulates the production of anthocyanin

20   pigments (red color) in plant tissues (Dellaporta *et al.*, Stadler Symposium *11*:263-282 (1988)); a

β-lactamase gene (Sutcliffe *et al., Proc. Natl. Acad. Sci. (U.S.A.) 75*:3737-3741 (1978)), a gene

which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a

chromogenic cephalosporin); a luciferase gene (Ow *et al., Science 234*:856-859 (1986)); a xylE

gene (Zukowsky *et al., Proc. Natl. Acad. Sci. (U.S.A.) 80*:1101-1105 (1983)) which encodes a

25   catechol dioxygenase that can convert chromogenic catechols; an α-amylase gene (Ikatu *et al.*,

Bio/Technol. 8:241-242 (1990)); a tyrosinase gene (Katz *et al., J. Gen. Microbiol. 129*:2703-

2714 (1983)) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone

which in turn condenses to melanin; an α-galactosidase, which will turn a chromogenic α-galactose substrate.

Included within the terms "selectable or screenable marker genes" are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins which are detectable, (e.g., by ELISA), small active enzymes which are detectable in extracellular solution (e.g., α-amylase, β-lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell wall (such as proteins which include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those of skill in the art.

There are many methods for introducing transforming nucleic acid molecules into plant cells. Suitable methods are believed to include virtually any method by which nucleic acid molecules may be introduced into a cell, such as by *Agrobacterium* infection or direct delivery of nucleic acid molecules such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, etc (Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol. 42*:205-225 (1991); Vasil, *Plant Mol. Biol. 25*:925-937 (1994)). For example, electroporation has been used to transform maize protoplasts (Fromm *et al., Nature 312*:791-793 (1986)).

Other vector systems suitable for introducing transforming DNA into a host plant cell include but are not limited to binary artificial chromosome (BIBAC) vectors (Hamilton *et al., Gene 200*:107-116 (1997)); and transfection with RNA viral vectors (Della-Cioppa *et al., Ann. N.Y. Acad. Sci.* (1996), 792 (Engineering Plants for Commercial Products and Applications), 57-61). Additional vector systems also include plant selectable YAC vectors such as those described in Mullen *et al., Molecular Breeding 4*:449-457 (1988)).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann,

5   *Biochem. Biophys. Res. Commun. 107*:584-587 (1982); Fromm *et al., Proc. Natl. Acad. Sci. (U.S.A.) 82*:5824-5828 (1985); U.S. Patent No. 5,384,253); and the gene gun (Johnston and Tang, *Methods Cell Biol. 43*:353-365 (1994)); (3) viral vectors (Clapp, *Clin. Perinatol. 20*:155-168 (1993); Lu *et al., J. Exp. Med. 178*:2089-2096 (1993); Eglitis and Anderson, *Biotechniques 6*:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al., Hum. Gen. Ther. 3*:147-

10   154 (1992), Wagner *et al., Proc. Natl. Acad. Sci. (USA) 89*:6099-6103 (1992)).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules to plant cells is microprojectile bombardment. This method has been reviewed by Yang and Christou (eds.), *Particle Bombardment Technology for Gene Transfer*, Oxford Press,

15   Oxford, England (1994)). Non-biological particles (microprojectiles) that may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum and the like.

A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of

20   protoplasts (Cristou *et al., Plant Physiol. 87*:671-674 (1988)) nor the susceptibility of *Agrobacterium* infection are required. An illustrative embodiment of a method for delivering DNA into maize cells by acceleration is a biolistics α-particle delivery system, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in suspension. Gordon-Kamm *et al.,*

25   describes the basic procedure for coating tungsten particles with DNA (Gordon-Kamm *et al., Plant Cell 2*:603-618 (1990)). The screen disperses the tungsten nucleic acid particles so that they are not delivered to the recipient cells in large aggregates. A particle delivery system

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suitable for use with the invention is the helium acceleration PDS-1000/He gun is available from Bio-Rad Laboratories (Bio-Rad, Hercules, California)(Sanford *et al., Technique 3*:3-16 (1991)).

For the bombardment, cells in suspension may be concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the

5   microprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded.

Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the

10 acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more foci of cells transiently expressing a screenable or selectable marker gene. The number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from one to ten and average one to three.

In bombardment transformation, one may optimize the pre-bombardment culturing

15 conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately

20 after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

In another alternative embodiment, plastids can be stably transformed. Methods

25 disclosed for plastid transformation in higher plants include the particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (Svab *et al., Proc. Natl. Acad. Sci. (U.S.A.)* 87:8526-8530 (1990);

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Svab and Maliga, *Proc. Natl. Acad. Sci. (U.S.A.) 90*:913-917 (1993); Staub and Maliga, *EMBO J. 12*:601-606 (1993); U.S. Patents 5, 451,513 and 5,545,818).

Accordingly, it is contemplated that one may wish to adjust various aspects of the bombardment parameters in small-scale studies to fully optimize the conditions. One may

5   particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance and helium pressure. One may also minimize the trauma reduction factors by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted

10   for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated

15   plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example the methods described by Fraley *et al., Bio/Technology 3*:629-635 (1985) and Rogers *et al., Methods Enzymol. 153*:253-277 (1987). Further, the integration of the T-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome as

20   described (Spielmann *et al., Mol. Gen. Genet. 205*:34 (1986)).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.,* In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985)). Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer

25   have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site

for direct expression of inserted polypeptide coding genes and are suitable for present purposes (Rogers *et al., Methods Enzymol. 153*:253-277 (1987)). In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of

5   the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. More preferred is a transgenic plant that is homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same

10   locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to

15   produce offspring that contain two independently segregating, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Backcrossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Transformation of plant protoplasts can be achieved using methods based on calcium

20   phosphate precipitation, polyethylene glycol treatment, electroporation and combinations of these treatments (*See, for example*, Potrykus *et al., Mol. Gen. Genet. 205*:193-200 (1986); Lorz *et al., Mol. Gen. Genet. 199*:178 (1985); Fromm *et al., Nature 319*:791 (1986); Uchimiya *et al., Mol. Gen. Genet. 204*:204 (1986); Marcotte *et al., Nature 335*:454-457 (1988)).

Application of these systems to different plant strains depends upon the ability to

25   regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al., Plant Tissue Culture Letters 2*:74

(1985); Toriyama *et al., Theor Appl. Genet.* 205:34 (1986); Yamada *et al., Plant Cell Rep. 4*:85 (1986); Abdullah *et al., Biotechnology* 4:1087 (1986)).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, *Biotechnology* 6:397 (1988)). In addition, "particle gun" or high-velocity microprojectile technology can be utilized (Vasil *et al., Bio/Technology 10*:667 (1992)).

Using the latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al., Nature 328:*70 (1987); Klein *et al., Proc. Natl. Acad. Sci. (U.S.A.)* 85:8502-8505 (1988); McCabe *et al., Bio/Technology 6:923* (1988)). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants are well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA, (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent No. 5,004,863; U.S. Patent No. 5,159,135; U.S. Patent No. 5,518,908); soybean (U.S. Patent No. 5,569,834; U.S. Patent No. 5,416,011; McCabe *et al., Biotechnology 6:*923 (1988); Christou *et al., Plant Physiol. 87:*671-674 (1988)); *Brassica* (U.S. Patent No. 5,463,174); peanut (Cheng *et al., Plant Cell Rep. 15:*653-657 (1996), McKently *et al., Plant Cell Rep. 14:*699-703 (1995)); papaya; and pea (Grant *et al., Plant Cell Rep. 15:*254-258 (1995)).

Transformation of monocotyledons using electroporation, particle bombardment and *Agrobacterium* have also been reported. Transformation and plant regeneration have been achieved in asparagus (Bytebier *et al., Proc. Natl. Acad. Sci. (USA) 84:*5354 (1987)); barley (Wan and Lemaux, *Plant Physiol 104:*37 (1994)); maize (Rhodes *et al., Science 240:*204 (1988); Gordon-Kamm *et al., Plant Cell 2:*603-618 (1990); Fromm *et al., Bio/Technology 8:*833 (1990); Koziel *et al., Bio/Technology 11:*194 (1993); Armstrong *et al., Crop Science 35:*550-557 (1995)); oat (Somers *et al., Bio/Technology 10:*1589 (1992)); orchard grass (Horn *et al., Plant Cell Rep. 7:*469 (1988)); rice (Toriyama *et al., Theor Appl. Genet. 205:*34 (1986); Part *et al., Plant Mol. Biol. 32:*1135-1148 (1996); Abedinia *et al., Aust. J. Plant Physiol. 24:*133-141 (1997); Zhang and Wu, *Theor. Appl. Genet. 76:*835 (1988); Zhang *et al., Plant Cell Rep. 7:*379 (1988); Battraw and Hall, *Plant Sci. 86:*191-202 (1992); Christou *et al., Bio/Technology 9:*957 (1991)); rye (De la Pena *et al., Nature 325:*274 (1987)); sugarcane (Bower and Birch, *Plant J. 2:*409 (1992)); tall fescue (Wang *et al., Bio/Technology 10:*691 (1992)) and wheat (Vasil *et al., Bio/Technology 10:*667 (1992); U.S. Patent No. 5,631,152).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al., Nature*

335:454-457 (1988); Marcotte *et al., Plant Cell 1*:523-532 (1989); McCarty *et al., Cell 66*:895-905 (1991); Hattori *et al., Genes Dev. 6*:609-618 (1992); Goff *et al., EMBO J. 9*:2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs (*see generally*, Mailga *et al., Methods in Plant Molecular Biology,* Cold Spring Harbor Press (1995)).

5   Any of the nucleic acid molecules of the invention may be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers, *etc.* Further, any of the nucleic acid molecules of the invention may be introduced into a plant cell in a manner that allows for overexpression of the protein or fragment thereof encoded by the nucleic acid molecule.

10   Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al., Plant Cell 2*:279-289 (1990); van der Krol *et al., Plant Cell 2*:291-299 (1990)). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found within the cell (Prolls and Meyer, *Plant J. 2*:465-475 (1992)) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found within the cell (Mittlesten *et al., Mol. Gen. Genet. 244*:325-330 (1994)). Genes, even though different, linked to homologous promoters may result in the cosuppression of the linked genes (Vaucheret, *C.R. Acad. Sci. III 316*:1471-1483 (1993);

20   Flavell, *Proc. Natl. Acad. Sci. (U.S.A.) 91*:3490-3496 (1994)); van Blokland *et al., Plant J. 6*:861-877 (1994); Jorgensen, *Trends Biotechnol. 8*:340-344 (1990); Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants,* Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994)).

It is understood that one or more of the nucleic acids of the invention may be introduced

25   into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous protein.

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Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (U.S. Patents 4,801,540 and 5,107,065 Mol *et al.*, *FEBS Lett. 268*:427-430 (1990)). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.,* In: *Genetic Engineering,* Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989)).

The principle of regulation by antisense RNA is that RNA that is complementary to the target mRNA is introduced into cells, resulting in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al.*, *Annu. Rev. Biochem. 55*:569-597 (1986)). Under one embodiment, the process involves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol. 25*:155-184 (1990)). An antisense vector is constructed by standard procedures and introduced into cells by transformation, transfection, electroporation, microinjection, infection, *etc.* The type of transformation and choice of vector will determine whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It is understood that the activity of a protein in a plant cell may be reduced or depressed by growing a transformed plant cell containing a nucleic acid molecule whose non-transcribed strand encodes a protein or fragment thereof.

Post transcriptional gene silencing (PTGS) can result in virus immunity or gene silencing in plants. PTGS is induced by dsRNA and is mediated by an RNA-dependent RNA polymerase, present in the cytoplasm, that requires a dsRNA template. The dsRNA is formed by hybridization of complementary transgene mRNAs or complementary regions of the same

5   transcript. Duplex formation can be accomplished by using transcripts from one sense gene and one antisense gene co-located in the plant genome, a single transcript that has self-complementarity, or sense and antisense transcripts from genes brought together by crossing. The dsRNA-dependent RNA polymerase makes a complementary strand from the transgene mRNA and RNAse molecules attach to this complementary strand (cRNA). These cRNA-

10   RNAse molecules hybridize to the endogene mRNA and cleave the single-stranded RNA adjacent to the hybrid. The cleaved single-stranded RNAs are further degraded by other host RNAses because one will lack a capped 5' end and the other will lack a poly(A) tail (Waterhouse *et al., PNAS 95*: 13959-13964 (1998)).

It is understood that one or more of the nucleic acids of the invention may be introduced

15   into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the postranscriptional gene silencing of an endogenous transcript.

Antibodies have been expressed in plants (Hiatt *et al., Nature 342:*76-78 (1989); Conrad and Fielder, *Plant Mol. Biol. 26:*1023-1030 (1994)). Cytoplasmic expression of a scFv (single-chain Fv antibodies) has been reported to delay infection by artichoke mottled crinkle virus.

20   Transgenic plants that express antibodies directed against endogenous proteins may exhibit a physiological effect (Philips *et al., EMBO J. 16:*4489-4496 (1997); Marion-Poll, *Trends in Plant Science 2:*447-448 (1997)). For example, expressed anti-abscissic antibodies have been reported to result in a general perturbation of seed development (Philips *et al., EMBO J. 16:* 4489-4496 (1997)).

25   Antibodies that are catalytic may also be expressed in plants (abzymes). The principle behind abzymes is that since antibodies may be raised against many molecules, this recognition ability can be directed toward generating antibodies that bind transition states to force a chemical

reaction forward (Persidas, *Nature Biotechnology 15*:1313-1315 (1997); Baca *et al., Ann. Rev. Biophys. Biomol. Struct. 26*:461-493 (1997)). The catalytic abilities of abzymes may be enhanced by site directed mutagenesis. Examples of abzymes are, for example, set forth in U.S. Patent No: 5,658,753; U.S. Patent No. 5,632,990; U.S. Patent No. 5,631,137; U.S. Patent

5   5,602,015; U.S. Patent No. 5,559,538; U.S. Patent No. 5,576,174; U.S. Patent No. 5,500,358; U.S. Patent 5,318,897; U.S. Patent No. 5,298,409; U.S. Patent No. 5,258,289 and U.S. Patent No. 5,194,585.

It is understood that any of the antibodies of the invention may be expressed in plants and that such expression can result in a physiological effect. It is also understood that any of the

10 expressed antibodies may be catalytic.

(d)   Antibodies

One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the present invention and their homologues, fusions or fragments. Such antibodies

15 may be used to quantitatively or qualitatively detect the protein or peptide molecules of the present invention. As used herein, an antibody or peptide is said to "specifically bind" to a protein or peptide molecule of the present invention if such binding is not competitively inhibited by the presence of non-related molecules.

Nucleic acid molecules that encode all or part of the protein of the present invention can

20 be expressed, via recombinant means, to yield protein or peptides that can in turn be used to elicit antibodies that are capable of binding the expressed protein or peptide. Such antibodies may be used in immunoassays for that protein. Such protein-encoding molecules, or their fragments may be a "fusion" molecule (*i.e.*, a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced. It is understood that any of the nucleic acid

25 molecules of the present invention may be expressed, via recombinant means, to yield proteins or peptides encoded by these nucleic acid molecules.

The antibodies that specifically bind proteins and protein fragments of the present invention may be polyclonal or monoclonal and may comprise intact immunoglobulins, or antigen binding portions of immunoglobulins fragments (such as (F(ab'), F(ab')$_2$), or single-chain immunoglobulins producible, for example, via recombinant means. It is understood that

5   practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example, Harlow and Lane, In: *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988)).

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for

10   this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 μg of purified protein (or fragment thereof) that has been emulsified in a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 μg of

15   antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-protein or peptide antibodies. Preferably, a direct binding Enzyme-Linked Immunoassay (ELISA) is employed for this purpose.

More preferably, the mouse having the highest antibody titer is given a third i.v. injection

20   of approximately 25 μg of the same protein or fragment. The splenic leukocytes from this animal may be recovered 3 days later and then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under "HAT" (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones

25   may then be screened for their capacity to produce monoclonal antibodies ("mAbs"), preferably by direct ELISA.

In one embodiment, anti-protein or peptide monoclonal antibodies are isolated using a

fusion of a protein or peptide of the present invention, or conjugate of a protein or peptide of the

present invention, as immunogens. Thus, for example, a group of mice can be immunized using

a fusion protein emulsified in Freund's complete adjuvant (e.g., approximately 50 µg of antigen

5   per immunization). At three week intervals, an identical amount of antigen is emulsified in

Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third

immunization, serum samples are taken and evaluated for the presence of antibody. If antibody

titers are too low, a fourth booster can be employed. Polysera capable of binding the protein or

peptide can also be obtained using this method.

10   In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-

described immunized mice are removed, disrupted and immune splenocytes are isolated over a

ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-

derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653

plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for

15   hybridoma fusion cells by their capacity to grow in culture medium supplemented with

hypothanthine, aminopterin and thymidine for approximately 2-3 weeks.

Hybridoma cells that arise from such incubation are preferably screened for their capacity

to produce an immunoglobulin that binds to a protein of interest. An indirect ELISA may be

used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells

20   that contain immobilized protein. After washing, the titer of bound immunoglobulin can be

determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase.

After additional washing, the amount of immobilized enzyme is determined (for example

through the use of a chromogenic substrate). Such screening is performed as quickly as possible

after the identification of the hybridoma in order to ensure that a desired clone is not overgrown

25   by non-secreting neighbor cells. Desirably, the fusion plates are screened several times since the

rates of hybridoma growth vary. In a preferred sub-embodiment, a different antigenic form may

be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with

one immunogen, but the resulting hybridomas can be screened using a different immunogen. It is understood that any of the protein or peptide molecules of the present invention may be used to raise antibodies.

Such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind the protein or peptide molecules of the present invention permits the identification of mimetic compounds of those molecules. A "mimetic compound" is a compound that is not that compound, or a fragment of that compound, but which nonetheless exhibits an ability to specifically bind to antibodies directed against that compound.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1

In this example, DNA is extracted from soybean plants, amplified, and mapped.

A single trifoliate leaf is collected from the newest growth of four week old soybean plants. Leaf tissue from the leaf is placed on ice and stored at -80°C. The frozen tissue is lyophilized, and approximately 0.01 grams of the tissue is used for DNA extraction. The 0.01 grams of leaf tissue is ground to powder in 1.4 ml tubes. 600 microliters (μl) of DNA extraction buffer consisting of 0.5M NaCl, 0.1M Tris-(hydroxymethyl) aminomethane pH 8.0, 0.05 M ethylenediaminetetra-acetic acid (EDTA), 10.0 g L^{-1} sodium dodecyl sulfate (SDS), and 2 g L^{-1} phenantroline (dissolved in 0.01 L ethanol) is heated to 65°C (with 0.77 g L^{-1} dithiothreitol added immediately before use) is added to each tube, and each tube is mixed thoroughly. The samples are placed in a 65°C water bath for 15 minutes and shaken by hand after 10 minutes. The samples are taken out of the water bath and cooled to room temperature, and then 200 μl of 5 M KOAc is added to each tube. The samples are inverted and placed at 4°C for 20 minutes.

Samples are then centrifuged for 12 minutes at 6200 X g and the supernatant (about 600 µl) is transferred to new tubes. DNA is precipitated with 330 µl of cold isopropanol and placed at -20°C for 1 hr. The DNA is pelleted by centrifuging at 6200 X g for 10 minutes and washed with 70% EtOH. The DNA is pelleted by centrifugation at 6200 X g for 10 minutes and dried using a

5   Speed-Vac. The DNA is dissolved in 100 µl of $TE_{0.1}$ (0.01 M Tris-HCl pH 8.0, 0.0001 M EDTA). The extraction will generally yield 500 ng DNA $µl^{-1}$.

A polymerase chain reaction (PCR) is conducted with 5 to 10 ng genomic DNA in 10 µl volumes of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 150 nM of each primer, 0.01 mM Cresol Red, 2% sucrose and 0.32 units of

10   AmpliTaq DNA Polymerase (Perkin Elmer Instruments Inc., USA). For thermocycling, the Gene Amp PCR System 9700 (Perkin Elmer Instruments Inc., USA) is used with one step of 94°C for 3 minutes, then 32 cycles of 94°C, 47°C, and 72°C steps of 25 sec each and one final step of 72°C for 3 minutes. The PCR products are run on a 6% polyacrylamide gel (30 cm X 8 cm X 1 mm) in 1X TAE (40 mM Tris-HCl, pH 8.3, 1 mM EDTA) at 180 v for 45 minutes. The

15   gels are stained using SYBR Gold (Molecular Probes, Eugene, OR) according to the manufacturer's instructions.

SSR primer screening for polymorphism is performed using PIC, HS-1, Will and PI507354 genotypes. SSRs that are polymorphic and easy to score (*i.e.*, clear banding pattern and good separation between alleles) are mapped using the HS-1 x PIC (F2) and/or Will x

20   PI507354 (RIL) mapping populations. At least one SSR per BAC sequence is mapped. DNA markers that exhibited codominant banding patterns are scored as homozygous for one or the other parent or as heterozygous, exhibiting both parental alleles. Marker scores are checked for segregation distortion using the chi-squared test for goodness of fit to expected ratios. Linkage relationships are determined using Mapmaker Version 3.0b with a LOD of 3.0 (Whitehead

25   Institute, Cambridge, MA).

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EXAMPLE 2

DNA fragments containing candidates for genes *rhg*1 and *Rhg*4 from susceptible and resistant soybean lines are subcloned into a TA cloning plasmid (TOPO TA Cloning Kit, Version E, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA).

5   Genomic DNA from 24 susceptible and 9 resistant lines is isolated using standard techniques. Approximately 500 nanograms (ng) of DNA is used for PCR amplification. Resistant BAC DNA is isolated by using AUTOGEN (AutoGen Corp., 35 Loring Drive Framingham, MA). PCR amplification is then performed using 0.1- 0.2 ng of resistant BAC DNA. The primers that are used to amplify candidate *rhg*1 genes PCR are as follows:

10   Fragment I (2,892 bp) primer (SEQ ID NO: 25), GCA ATA CTT GAA GGA ATA TGT CCA C; primer (SEQ ID NO: 24), beginning at start codon, ATG GAT GGT AAA AAT TCA AAA CTA AAC; modified reverse primer 1 (SEQ ID NO: 1123), beginning 5 bp before start codon; GTT GTA TGG ATG GTA AAA ATT CAA AAC. Fragment II (1,746 bp) reverse primer 2 (SEQ ID NO: 27), ending at 13 bp after stop codon, GAC TGG CTG TGA CTG ATC TCT CT; primer 2 (SEQ ID NO: 26), CTC ACT TAC ACT GCT GAA TGC AGA.

15   The primers for *Rgh4* PCR are as follows:

Forward primer (SEQ ID NO: 48), ATG TCT CTC CCC AAA ACC CTA CTT TCT CTC; reverse primer (SEQ ID NO: 49), ending at 2 bp after stop codon, GGT TAA CGG CAA TCC ATT GAA TCA AAG GAG.

20   PCR amplification is performed in an MJ Research PTC DNA Engine *TM* System, Model PTC-225 (MJ Research Inc, 590 Lincoln Street Waltham, MA). PCR is performed using the following components: 1μl DNA, 5μl 10x buffer, 1μl primer 1, 1μl primer 2, 1μl 10mM dNTP, 1.5 μl 50mM MgCl$_2$, 0.2μl Taq. (Platinum), 39.3μl H$_2$O. The PCR program used is as follows: 95°C for 10 minutes (step 1), 95°C for 30 seconds (step 2), 70°C for 30 seconds/-1°C per

25   cycle/72°C for 3 minutes (step 3), repeat steps two through three 9 times (step 4), 95°C for 30 seconds (step 5), 60°C for 30 seconds (step 6), 72°C for 3 minutes (step 7), repeat steps five through seven 34 times (step 8), 4°C forever (step 9), end.

PCR products are separated on 1% agarose gel by electrophoresis. A single DNA band is excised from gel. Gel extraction is done using CLONTECH NucleoSpin Extraction Kit (Clonetech Laboratories Inc., 1020 East Meadow Circle, Palo Alto, CA). 2 μl of purified DNA is loaded on 1% agarose gel to check concentration. 40-100ng of DNA is used for subcloning.

5   A TOPO cloning reaction is done according to the following: 4μl of fresh PCR product, 1μl Clontech Salt Solution, and 1 μl TOPO vector. The solution is mixed gently, incubated for 10 minutes at room temperature, and then placed on ice.

A one shot chemical transformation is performed as follows. 2μl of the TOPO Cloning reaction is added to a vial of TOP 10 One Shot Chemically Competent *E. coli* and mixed gently.

10   The mixture is then Incubated on ice for 30 minutes. The cells are then heat-shocked for 30 seconds at 42°C, and immediately transferred to ice. 250 μl of SOC medium is then added, and the mixture is incubated at 37°C for 1 hour. 80 μl is then spread onto a selective plate, and 170 μl is spread onto another plate. The plates are incubated at 37°C for 18-20 hours. The selective plates are LB agar plates with 100 μg/ml ampicillin, 40 μg/ml IPTG, and 40 μg/ml X-GAL.

15   After incubation, 8-10 white or light blue colonies are selected. The positive colonies are inoculated into LB medium containing 50 μg/ml ampicillin and incubated at 37°C overnight. Sterilized glycerol is added to make 15% glycerol stock, which can be stored at -80°C.

Sanger sequencing reactions are performed on subclones using BigDye Terminators (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA) and then analyzed on ABI

20   377/ABI 3700 automated sequencing machines (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA). The sequences are evaluated for quality and error probability using the program, PHRED (Ewing and Green, Genome Res., 8:186-194 (1998), Ewing *et al.*, Genome Res., 8:175-185, (1998)), assembled using the *phrap* assembler and viewed using *consed* (Gordon *et al.*, Genome Res., 8:195-202). An *rhg*1 candidate gene is found in BAC 240O17,

25   and is about 4.5 kb in size. An *Rhg*4 candidate was found in BAC 318O13, and is about 3.5 kb in size.

EXAMPLE 3

The physical mapping of a QTL (quantitative trait locus) is described in this example.
Mapping is initiated with linkage analysis of SSR (simple sequence repeats) markers. Markers
that are shown to be linked to the QTL of interest are used to PCR screen the soy BAC library
and identify candidate BACs. Confirmed BACs are subcloned and sequenced, BAC-end
sequenced, and fingerprinted. New markers are designed from good BAC-end sequences and
used to screen the library, by either PCR or hybridization to high density grid filters, in order to
extend the contigs. A BAC-end sequence and fingerprint database of soy BACs is used in
conjunction with the above methods to help build and extend contigs. Sequenced BACs are
aligned, and overlapping BACs are placed into contigs. These contigs, which contain unique
sequences, are put into an ACEDB database, and predicted genes are annotated by hand using
various programs. Candidates genes (for the gene of interest) are subcloned from genomic DNA
of different lines by PCR using primers from outside the predicted coding regions. These
subclones are sequenced and screened for SNPs (single nucleotide polymorphisms) and INDELs
(insertions/deletions), and different haplotypes of the lines with and without the desired
phenotype are examined for correlations between the haplotype and phenotype.

A single trifoliate leaf is collected from the newest growth of four week old soybean
plants. The leaf tissue is placed on ice and stored at -80°C. The frozen tissue is lyophilized and
approximately 0.01 grams of tissue is used for DNA extraction. The leaf tissue is ground to
powder in 1.4 ml tubes and 600 µl of DNA extraction buffer [0.5M NaCl, 0.1M Tris-
(hydroxymethyl) aminomethane pH 8.0, 0.05 M ethylenediaminetetra-acetic acid (EDTA), 10.0
g L^{-1} sodium dodecyl sulfate (SDS), 2 g L^{-1} phenantroline (dissolved in 0.01 L ethanol)] heated
to 65°C (with 0.77 g L^{-1} dithiothreitol added immediately before use) is added to each tube and
mixed thoroughly. The samples are placed in a 65°C water bath for 15 minutes and shaken by
hand after 10 min. The samples are taken out of the water bath, cooled to room temperature, and
200 µl of 5 M KOAc is added to each tube. The samples are inverted and placed at 4°C for 20
min. Samples are then centrifuged for 12 minutes at 6200 X g and the supernatant (about 600 µl)

is transferred to new tubes. DNA is precipitated with 330 µl of cold isopropanol and placed at -20°C for 1 hr. The DNA is pelleted by centrifuging at 6200 X g for 10 minutes and is washed with 70% EtOH. The DNA is pelleted by centrifugation at 6200 X g for 10 minutes and dried using a Speed-Vac. The DNA is dissolved in 100 µl of $TE_{0.1}$ (0.01 M Tris-HCl pH 8.0, 0.0001

5   M EDTA). The extraction yields 500 ng DNA μl^{-1}.

The polymerase chain reaction (PCR) is conducted with 5 to 10 ng genomic DNA in 10 µl volumes of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 150 nM of each primer, 0.01 mM Cresol Red, 2% sucrose and 0.32 units of AmpliTaq DNA Polymerase (Perkin Elmer Instruments Inc., USA, 761 Main Avenue, Norwalk,

10   CT). For thermocycling, the Gene Amp PCR System 9700 (Perkin Elmer Instruments Inc., USA, 761 Main Avenue, Norwalk, CT) is used with one step of 94°C for 3 min, then 32 cycles of 94°C, 47°C and 72°C steps of 25 sec each and one final step of 72°C for 3 min. The PCR products are run on a 6% polyacrylamide gel (30 cm X 8 cm X 1 mm) in 1X TAE (40 mM Tris-HCl, pH 8.3, 1 mM EDTA) at 180v for 45 min. The gels are stained using SYBR Gold

15   (Molecular Probes, Eugene, OR) per manufacturers instructions.

SSR primer screening for polymorphisms is performed using PIC, HS-1, Will and PI507354 genotypes. SSRs that are polymorphic and easy to score (i.e., Clear banding pattern and good separation between alleles) are mapped using the HS-1 x PIC (F2) and/or Will x PI507354 (RIL) mapping populations. At least one SSR per BAC sequence is mapped. DNA

20   markers that exhibited codominant banding patterns are scored as homozygous for one or the other parent or as heterozygous, exhibiting both parental alleles. Marker scores are checked for segregation distortion using the chi-squared test for goodness of fit to expected ratios. Linkage relationships were determined using Mapmaker Version 3.0b with a LOD of 3.0 (Whitehead Institute for Biomedical Research, Cambridge MA).

25   Thirty-two BAC DNA superpools (10 genomic equivalents) extracted from either 4608 clones (48 96-well microtiter plates) are used as templates for the first round of PCR screening. Following identification of the positive superpools, the second screening is performed against 4-

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D BAC DNA pools. Each clone of the superpool is addressed 4-dimentionally (7 X 7 X 12 X 8) and pooled in each dimension. Each set of 48 plates is divided into 6 sets of 7 plates and one set of 6 plates, and partitioned in two ways. The first partition is in numerical order, plates 1-7, 8-14, ... 43-48 representing 7 group or stack pools. The second partition is according to plate

5   position within each of the respective stacks, plates [1, 8, 15, 22, 29, 36], [2,9,16, 23, 30, 37, 43] *etc.*, representing 7 plate pools. Each well of the 96-well plates contains 12 columns and 8 rows. Clones from row 1 are pooled from all 48 plates to generate the row 1 pool. Clones of rows 2, 3, 4....8, and columns 1, 2, 3....12 are pooled to generate 8 row pools and 12 column pools respectively.

10   For each superpool, BAC DNA is extracted from a total of 34 subpools (7 + 7 + 8 + 12). Positive clones are identified by TaqMan/PCR screening of the 34 subpools if one positive clone is present. If more than one positive clone is present in a superpool, a third round of screening with N4 PCR reactions is performed.

Addresses of candidate BACs are identified, and the candidates are streaked out for

15   single colony isolation and grown overnight at 37°C. A single, isolated colony is picked and streaked out and grown overnight at 37°C. PCR is repeated for the marker of interest (using the program designed for the relevant marker) using a smear of cells from the plate streaked from a single colony. The PCR product is run on a 2% agarose gel and purified using the Clonetech NucleoSpin Gel Extraction Kit (according to the manufacturer's instructions, Clonetech

20   Laboratories Inc., 1020 East Meadow Circle, Palo Alto, CA) and 10-50 ng of the purified DNA are added to 10 pmol of each primer (forward and reverse), in a total volume of 6 µl of ddH2O and 2 µl of BigDye Terminators (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA). The cycling conditions are: 96°C for 1 minute (step 1), 96°C for 10 seconds (step 2), 50°C for 5 seconds (step 3), 60°C for 4 minutes (step 4), steps 2-4 are repeated for 24 cycles (step 5),

25   and hold at 4°C.

The generated sequence is compared to the consensus sequence using DNA comparison software. Confirmed clones are subcloned, sequenced, BAC-end sequenced, and Fingerprinted.

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BAC-end sequencing is done using 3.2 pmol of SP6 and T7 primers (separately), approximately 600 ng-1 ug of BAC DNA (Autogen prepped, AutoGen Corp., 35 Loring Drive Framingham, MA) reaction, resuspended in 6μl of ddH2O, and 4μl of BigDye Terminators (Applied Biosystems 850 Lincoln Centre Drive, Foster City, CA) to give a total reaction volume

5   of 10ul. The cycling conditions are: 96°C for 2 minutes (step 1), 96°C for 15 seconds (step 2), 50°C for 15 seconds (step 3), 60°C for 4 minutes (step 4), steps 2-4 are repeated for 50-60 cycles (step 5), 72°C for 2 minutes (step 6), hold at 4°C or 10°C (step 7).

The reactions are ethanol precipitated and loaded on capillary sequencers. The newly generated BAC-end sequence is trimmed from the vector sequence, and entered into a database

10   containing approximately 400,000 BAC-end sequences. Each BAC is blasted against the database to search for BAC-end matches extension of the contigs. New markers are designed from good BAC-end sequences, and these are then used to rescreen the library in order to build up contigs across the region of interest. Screening can be done in either of two ways: as above (PCR strategy), or by hybridization of high-density grid filters from Research Genetics

15   (Research Genetics, 2130 Memorial Parkway, Huntsville, AL).

The probes used for hybridization are derived from clones or genomic DNA by PCR amplification using the vector or gene-specific primers, with the appropriate cycling conditions. PCR products are run on a 1% agarose gel containing ethidium bromide (0.2 ug/ml) in 1X TAE buffer at 100 volt for 1-2 hrs. Isolated DNA fragments are excised and gel-purified using the

20   Clonetech NucleoSpin gel extraction kit (Clonetech Laboratories Inc., 1020 East Meadow Circle, Palo Alto, CA), before labeling. In order to check the size of the fragments and concentration, 2 μl of eluted DNA plus loading buffer are loaded on a 1% agarose gel along with DNA markers of known concentration and size. All the probes used to screen the library are tested individually for repetitiveness, with a smaller filter spotted with random clones from the library along with

25   some positive control clones according to the protocol described below.

The A3244 soy library generated by a an *Eco*RI digest is spotted on 3 high density grid filters from Research Genetics (Research Genetics, 2130 Memorial Parkway, Huntsville, AL).

Each filter has six fields, twelve 384 well plates are spotted in each field in duplicate, with a total of 27,648 clones spotted on each filter. The plates are spotted in a 5X5 grid (12 clones per 5X5 grid) pattern within each field. Each clone is spotted in duplicate with a specific orientation within the 5X5 grid, which, together with the field position, gives information about its address.

5   In a first round hybridization procedure, multiple probes are labeled separately and then pooled together to hybridize to BAC filters. Positive BACs identified in this procedure are deconvoluted by rehybridization with the individual probes.

A hybridization oven is set at 65°C, and Church Buffer (0.5 M Sodium Phosphate, pH 7.0, 7% SDS, 1% bovine serum albumin, 1 mM EDTA, 100 μg/ml salmon sperm DNA) is

10   prewarmed to 65°C. Membranes are washed in 500 ml of 0.1X SSC, 0.1% SDS in a large container at room temperature for 5 minutes with gentle shaking (50 rpm) on a rotary shaker. The membranes are rinsed with 500 ml of 0.1X SSC (no SDS) for 1 minute. The wash solution is poured off, and 500 ml of 6XSSC (no SDS) is added to equilibrate the membranes. Three filters are placed in a tube. The filters are separated from each other and the sides of the tube by

15   a layer of mesh. Each tube is filled with 6XSSC and shaken gently with the tube vertical to help eliminate bubbles between the filters and tube wall. The 6X SSC solution is poured off, and 25 ml of pre-warmed Church buffer is added. The bottles are rotated in a hybridization oven at 60 rpm and 65°C for 30 minutes or longer.

Probes are labeled using 1 μl of 40-50 uCi/μl [α^{32}P dCTP], 50 ng of purified DNA in 49

20   μl of ddH2O, and Read-To-Go Labeling Beads from Amersham Pharmacia according to the manufacturers instructions (Amersham Pharmacia, Uppsala, Sweden). The probes are purified using the Bio-Spin Column P30 from BioRad according to manufacturers instructions (Bio-Rad Laboratories, 3316 Spring Garden Street, Philadelphia, PA). To 1 μl of the column-purified probe is added to a minipoly-Q vial (liquid scintillation vial) for each probe. 5 ml of scintillation

25   liquid is added to each vial, and radiation activity for each vial is measured using a liquid scintillation counter.

After the probes are purified and counted for radioactivity, 10-20 probes and one control probe (from 50 μl reaction) are pooled with 10^7 cpm/probe each, into one 1.5 ml eppendorf tube. The pooled probes are denatured at 99°C in a sand heating block for 10 minutes. The tubes are cooled on ice or ice water about 2 minutes, and then spun down at 14,000 rpm for 30 seconds in

5   microcentrifuge. The tubes are pre-hybridized in 25ml of Church buffer for at least 30 minutes, which is then poured off. 40 ml of fresh hybridization solution (pre-warmed Church buffer) is added. The pooled-probe solution is added to the hybridization tube. The tube is rotated in the hybridization oven at 60 rpm, 65°C overnight.

The probe solution is poured off, 30 ml of pre-warmed (65°C) 1X SSC, 0.1% SDS

10   washing solution is added to the hybridization tube, the hybridization tube is rotated in the hybridization oven (at 65° C) for 15 minutes, and the process is repeat two times. At the last wash, the tube is rotated 180° and at the same speed for 15 minutes at 65°C. The washing solution is poured off, and 2X SSC (no SDS) is added.

Excess liquid is removed from each filter by placing the filter on a piece of 3MM paper.

15   The washed filter is placed on developed film with the DNA-side up (the side BACs were spotted on), covered with Saran wrap, and squeezed to force out liquid and bubbles. The Saran wrap is folded to the other side of the film, fixed it with tape, and then dried Kimwipes. The wrapped filters are placed into a film cassette with the DNA-side up (the side BACs were spotted on), which is placed on BioMax MS film (Biomax Technologies Inc., Vancouver, BC, Canada)

20   in a darkroom, and exposed overnight at room temperature without an intensifying screen. Film is developed with a film developer in the dark room the next day, and each film is labeled with filter number, probe used for hybridization, exposure time, and date.

Starting from Field 3, a 384-well grid is put on the field with the A1 position of the grid on the upper right, and the grid is aligned to the image. The row and column position for each

25   positive clone on the BAC recording spreadsheet is determined and recorded. The pattern of the hybridization signal is matched to known patterns. There are 6 plate reference numbers for each of twelve patterns, which are arranged in the same manner as the 6 fields. Based on the signal

pattern and field number, a plate reference number is determined for each positive clone. The

grid is moved to the next field and the process is repeated. The original plate number (P) is

determined using the following formula: $P = (N-1) \times 72 + R$, where N is the filter number on

which the identified clone is present and R is the plate reference number previously determined.

5   The complete address of the identified clone is given by the original plate number plus its

position on the plate determined previously. BACs' addresses are identified and converted to

"imp" files according to a Q-bot file format.

24 working plates are loaded into a Q-bot (Genetix, Queensway, New Milton,

Hampshire, United Kingdom) 6-high hotel and media-filled 96-well plates are placed on the

10   deck. The Q-bot is run following the standard manual using the program called "Rearraying98"

with the settings given in Appendix III of the accompanying manual: BAC-Picking. Plates

containing picked clones are placed in a shaker incubator and grown overnight at 37° C at 200

rpm.

35 µl DNA solution are transferred from 96-well plates into a 384-well plate using a

15   Platemate such that 4 96-well plates of DNA are combined into one 384-well plate. The 384-pin

head (puck) is washed in 10% SDS solution for 5 minutes, ultrasonicated in a water bath for 3

minutes, washed with 70% ethanol for 1 min., and air dried for 3 minutes. The 384-well DNA

source plates and membranes are arranged on the deck according to the instruction from the

manual and the spotted grid design chosen for the membrane. Spotting pattern are designed so

20   that there is one control probe at each of the 4 corners of the membrane. An asymmetric pattern

is used to orient filters. The control probe concentration is about 5 ng/ul. Zeus is run according

to instructions. If the DNA concentration is lower than 5 ng/ul, the Zeus is run a second time to

double the amount of spotted DNA on the membrane. One of the empty spots is spot dyed, if

available, using one 384-well dye plate. If an empty spot is not available, it is printed on one of

25   the DNA spots. This spot marks the position for cutting filters into small membranes (9X12 cm).

Membranes are interleaved between 3M papers and left to air-dry. Each corner of each

membrane is marked with a permanent marker and numbered. Filters are denatured on the

surface of 3M paper soaked with denaturalization solution for 4 minutes, and then neutralized on the surface of 3 M paper soaked with neutralization solution for 5 minutes. The filters are washed with 2XSSC for 5 minutes and then air dried. The filters are then baked at 80°C for 1 hr. and cut into individual small membranes (9X12 cm) according to the marked corner.

5   To confirm and deconvolute, hybridizations are done as before, but with newly generated filters, and each probe is done separately with a single filter using the smaller tube. 15 ml of Church buffer is used for the hybridization.

Fingerprints are generated by digesting the BAC DNA with Hind III for 3 hours at 37°C and running the reaction on a 0.8% gel at 200V for 19 hours. The gels are stained with

10   SybrGreen, while shaking at room temperature for 45 minutes, and scanned with a FlourImager. The bands are sized using Frag software and the fingerprints are assembled into contigs within FPC. Every time new clones are added the contigs are rebuilt using a tolerance of 10 and a cutoff of 10^{-9}.

Subclones are generated and Sanger sequencing reactions were performed on randomly

15   chosen subclones using BigDye Terminators (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA) then analyzed on ABI 377/ABI 3700 automated sequencing machines (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA). 7-8 fold sequence coverage is thereby generated across the BAC. The sequences are evaluated for quality and error probability using the program, *phred*, assembled using the *phrap* assembler, and viewed using *consed*, as in

20   example 2. For Bermuda standard BACs, all contigs are ordered and oriented and all gaps are closed using a directed primer walking strategy. A final quality value of phred40 (1 base error in 10,000 bases) with no gap regions, double coverage or two chemistries across single stranded areas is achieved.

The sequence contigs are put into an ACEDB database along with soy EST and plant

25   EST matches, along with Blastx, Tblastx, and Plant Blastn hits. Genemark.hmm is used to predict possible genes, and GeneFinder is used to predict splicing sites, ORFs, potential coding regions, as well as start and stop codons. The contigs are then annotated by hand and predicted

genes accepted, edited, and modified based on the characteristics present in the sequence and matches to protein, nucleotide, and EST databases.

The high-density BAC library membranes used for hybridization are made by Research Genetics (Research Genetics, 2130 Memorial Parkway, Huntsville, AL), using a modified Q-bot

5   (Genetix, Queensway, New Milton, Hampshire, United Kingdom), 384-well plates containing BACs are spotted onto 22 cm X 22 cm Hybond N+ membranes (Amersham Pharmacia, Uppsala, Sweden). Bacteria from 72 plates are spotted twice onto one membrane, giving 55,296 colonies in total, or 27,648 unique clones per membrane. The plates are spotted into six "fields" per membrane, with each field having 12 plates spotted in duplicate. This spotting format results in

10   six fields with 384 grids in each field. Each grid is a 5X5 matrix containing 12 unique clones in duplicate, with the center position left empty. The two positions occupied by each clone in duplicate are designed to give a unique pattern that indicates the plate location of each clone. After spotting, the bacteria on the membrane are incubated for 8 hours on LB-agar plates containing 12.5 ug/ml chloramphenicol. The membranes are then denatured, neutralized,

15   washed in a standard procedure, UV-light crosslinked, and air-dried. The membranes can be stored and shipped at room temperature.

Every reference, patent, or other published work cited above is herein incorporated by reference in its entirety.